WHAT LIES BENEATH—DISCOVERING THE HIDDEN STORY OF PLANT ROOTS: ROOT GROWTH STRATEGIES OF WETLAND PLANTS IN NORTHERN ONTARIO ALONG A GRADIENT OF GROWING SEASON LENGTH

by

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Abstract

Root growth strategy can be classified as multi-season (winter-surviving) or single-season (fall-senescing). This study investigated the relationship of wetland plant species with multi-season roots (sMSR) and species with single-season roots (sSSR) to soil temperature across a climatic growing season gradient. Study sites were in Lake Superior Provincial Park and Manitoulin Island. In fall 2013, root ingrowth cores were installed in soil. Root length and shoot dry mass were measured at harvests from spring 2014 to spring 2015. Results showed that soil and air temperature differed by geographic zone; Lake Superior Provincial Park demonstrated lower temperatures than Manitoulin Island. Soil temperature affected root length (positively) and shoot dry mass (negatively). Root growth strategy had a nearly significant relationship with ratio of spring to maximum shoot dry mass—greater for sMSR than sSSR; and a significant relationship with relative late season shoot dry mass change—decreasing for sMSR and increasing for sSSR.

Keywords: root length, root growth strategy, multi-season, single-season, phenology, temperature, monocots, graminoids, forbs, *Cyperaceae, Sparganiaceae, Alismataceae*, wetlands, Lake Superior Provincial Park, Manitoulin Island.

Co-Authorship Statement

This research focuses on the root strategies of wetland plants in Northern Ontario. The research question was developed by Dr. Peter Ryser at Laurentian University, Department of Biology. Dr. Ryser chose the study species. I chose specific field locations and carried out the project, from field installation to harvests and data collection. I conducted the statistical analyses and wrote this thesis manuscript under the guidance of Dr. Ryser.

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OR: MASTERING THE ART OF UNDERWATER KNOT TYING

Preface

The present research focuses on root strategies of wetland plants in response to the growing season. Field sites were primarily in Ontario Provincial Parks. As a biologist, naturalist, and teacher, I recognize the value of learning about, advocating for, and protecting these essential ecosystems and their astonishing biodiversity.

"Wetlands are vital for human survival. They are among the world's most productive environments; cradles of biological diversity that provide the water and productivity upon which countless species of plants and animals depend for survival" (Ramsar Convention Secretariat 2014).

Lawren S. Harris
Canadian, 1885-1970
Beaver Swamp, Algoma, 1920
Oil on canvas 120.7 x 141.0 cm

Art Gallery of Ontario
Gift of Ruth Massey Tovell, Toronto, in memory Harold Murchison Tovell
1953.53/12

The painting shown here, with permission from the Art Gallery

of Ontario, is by Group of Seven member Lawren Harris. It is called *Beaver Swamp, Algoma* and was exhibited in Toronto in 1921. Responding to it, art critic Hector Charlesworth wrote that the painting of the swamp was a "repulsive, forbidding thing. One felt like taking a dose of quinine every time one looked at it. If ugliness is real beauty they have yet to prove it to a very large mass of the assembled public."

In the words of Canada's National Wetlands Working Group (1997), "This critic's comments reflect the mood of a bygone era. In Canada and globally we now recognize the swamp, as a picture or in nature, as a thing of beauty. The swamp in both forms has accrued in value with the passage of time. Unlike most ecosystem types, wetlands occur throughout Canada. As such, they have become part of our universal discussions on ecosystem concepts and relationships. Wetlands in the Arctic, Prairie and Boreal ecozones of Canada engender thoughts of specific places, peculiar flora and fauna, and a definitive biological setting often cherished by our citizens. These wetlands provide threads that wind their way through all our ecosystems."

My research seeks to understand influences on plant roots that wind their way through wetlands.

Chapter 1: Introduction

The National Wetlands Working Group (1997) defines a wetland as land saturated with water in sufficient quantity to sustain biological activity and other processes adapted to an aquatic environment, with poorly drained soils and aquatic vegetation. Given the continuing global decline in wetlands, despite the growing evidence of their importance to global biodiversity and ecological integrity (Ramsar Convention Secretariat 2014), it is essential that wetland research be conducted.

In light of the considerable role of roots in carbon and nutrient cycles and the role of vegetation turnover in future climate-induced changes in carbon uptake (Hendrick and Pregitzer 1993, Ahlström et al. 2015), there is a need to deepen understanding of root strategies and fine root dynamics (Radville et al. 2016). There is increasing evidence that root lifespan in the temperate wetlands of northern Ontario can be classified as using one of two strategies—over-wintering or multi-season roots versus that of fall-senescing or single-season roots (Ryser and Kamminga 2009). Multi- and single-season root growth strategies can be assumed to be comparable with the evergreen and deciduous leaf growth strategies of plants, which are known to be a factor in species distribution (Wright et al. 2004; Kikuzawa and Lechowicz 2011) and are important for adaptation to environmental changes (van Ommen Kloeke et al. 2012).

The present research investigated whether wetland plant species with multi-season roots (sMSR) and species with single-season roots (sSSR) respond to growing season length in a similar manner as species with evergreen leaves and species with deciduous leaves, respectively. This

introduction to root growth strategies begins with the contribution of roots to global ecological processes; followed by shoot and root growth economics, phenology, lifespan, and strategy; and lastly an overview of the present research and its significance.

1.1 Ecosystem effects of root growth and turnover

1.1.1 Primary productivity

Approximately one third of the world's net primary productivity is attributed to fine root production and turnover, an estimate that is considered to be conservative (Jackson et al. 1997). Other reviews of plant resource allocation state that global root production accounts for up to 67% of terrestrial net primary productivity (Abramoff and Finzi 2015). In temperate regions, root production is thought to account for 50% of stand production (Ruess et al. 2003) and up to 90% of litter production (Steinaker and Wilson 2005). In arctic ecosystems, it has been found that more than 80% of biomass in trees, shrubs, and grasses is produced belowground (Blume-Werry et al. 2016). In many ecosystems, roots account for more than 50% of net primary production, yet root growth remains much less studied than shoot growth (Sloan et al. 2016).

1.1.2 Carbon cycling

Root systems contribute substantially to the terrestrial carbon cycle and constitute a significant carbon sink (Lukac 2012, Silver and Miya 2001). The quantity of recycled carbon and nutrients in soil from roots may be greater than that from leaves in many forest ecosystems (Hendrick and Pregitzer 1993). A large proportion of global carbon is allocated to fine root production in plants

(Hendrick and Pregitzer 1993). Specifically, carbon from fine roots accounts for more than 5% of global atmospheric carbon (Jackson et al. 1997).

In their review of studies concerning the role of roots and soil microbes in soil respiration, Hanson et al. (2000) found that root and rhizosphere respiration can account for 10% to more than 90% of total soil respiration, depending on vegetation type and time of the year. This is in agreement with findings from Bazzaz (1990), who states that as much as 90% of CO_2 from the soil environment is due to the respiration of roots and rhizomes.

In their study of root structure and function of herbaceous, graminoid, and shrub species, Roumet et al. (2016) found that fine roots (≤ 2 mm diameter) play a primary role in the carbon economy, with approximately 40% of the net carbon fixed by photosynthesis allocated to fine roots. In a study of relationships between fine roots and ecosystem carbon cycling in black spruce forests in Alaska, it was found that root life cycles are more variable than aboveground processes, and that carbon and nutrients may cycle through roots at rates that are several orders of magnitude greater than for aboveground material (Ruess et al. 2003).

Root growth strategies (i.e., the production of multi- or single-season roots) could influence carbon cycling in the same manner as leaf growth strategies (Zak et al. 2000, Arora and Boer 2005, Anderson-Teixeira et al. 2013), in which deciduous (fall-senescing) or evergreen (overwintering) leaves are produced. In a screening of root overwintering strategies of perennial wetland monocots in northern Ontario, Tim Nieman, Yvonne Hoogzaad, Elize Marcotte, and Peter Ryser (unpublished results) found that 17 species possessed winter-surviving or multi-season roots, while 7 species possessed fall-senescing or single-season roots.

1.1.3 Climate change

In their analysis of the seasonal cycle of atmospheric CO_2 as it relates to variation in the activity of northern vegetation, Keeling et al. (1996) proposed that recently observed increases in the amplitude of this cycling reflect increasing CO_2 uptake by land plants, due to climate change and higher temperatures. In a review of ecosystem response to the global increase in carbon dioxide, Bazzaz (1990) indicated that in shrub species, increased CO_2 prompted increased root to shoot ratios in *Abutilon theophrasti* (*Malvaceae*) and a decrease in *Amaranthus retroflexus* (*Amaranthaceae*); that high CO_2 nearly doubled root to shoot ratios in four tree species of the genus *Betula*; and that root growth increased under higher CO_2 in the tree species *Quercus alba* grown in nutrient-limited conditions. It is thought, then, that root production might increase in response to increased atmospheric CO_2 content.

1.1.4 Phenology

As roots may respond to climate change in a different manner than shoots, we must improve our understanding of how climate change will influence whole-plant phenology (Radville et al. 2016). Phenology is defined as the effect of seasonal changes on biological phenomena. In a study of the phenology of fine roots and leaves in forest and grassland, fine roots accounted for 80-90% of total litter production (Steinaker and Wilson 2008). In a study of above- and below-ground plant production in three arctic plant communities along an elevation gradient, the duration of root production was approximately 50% greater than that of shoot production, which suggests that models of vegetation production may be incompletely represented when based only

on shoot production (Blume-Werry et al. 2016). In two graminoid species from different temperate ecosystems—*Poa pratensis* in a dry, old field and *Carex rostrata* in a sedge wetland—approximately 30% of early spring shoot growth was attributed to resources captured below-ground (root dry mass), highlighting the importance of the transfer of food resources to above-ground biomass (Bernard 1974).

In a review of root phenology studies, relationships between aboveground plant material and temperature were demonstrated, but comparable study belowground is lacking because factors influencing root phenology are less understood (Radville et al. 2016). Given widespread interest in predicting ecosystem responses to climate change, it is important to understand root growth and correlations between root and leaf phenology, especially since the timing of soil carbon flux will shift as vegetation patterns change (Blume-Werry et al. 2016; Sloan et al. 2016). There is an overall lack of understanding of phenological effects on roots, compared to what is presently known about above-ground plant phenology (Abramoff and Finzi 2015). A review of studies on plant roots should therefore begin with a survey of literature pertaining to shoots.

1.2 Shoot growth economics and phenology

Economic terms such as costs and benefits are often used in studies of plant resource allocation in reference to the allocation of energy and biomass inherent to plant physiological processes. Botanists have drawn parallels between the activities of plants and commercial businesses, citing the need for both to acquire resources that can be stored or spent, with the purpose of allocating resources to specific products (e.g. leaves and roots), which can themselves acquire further

resources (Bloom et al. 1985). Similarly, plants and businesses must be adaptable, and have the capacity to be ready for both immediate and long-term needs, and only continue to allocate energy to certain functions as long as marginal revenue is greater than marginal cost (Bloom et al. 1985).

With respect to plant adaptation to local conditions, in a study of the relationship between summer temperature and standing crop in North American and European sedge meadows, above-ground biomass was positively correlated with highest mean monthly temperature (Gorham 1974). In many ecosystems, there is a favourable period (i.e., functional growing season) of a plant's annual cycle during which it accumulates carbon; conversely, during an unfavourable period, which can result from factors such as cold or drought, photosynthesis does not occur (Kikuzawa and Lechowicz 2011). Plants from sites with a shorter favourable period have been found to have a higher concentration of nitrogen in their leaves, which could indicate higher rates of photosynthesis (Kikuzawa et al. 2013). In areas with a longer favourable period, deciduous leaves live longer compared to those in areas with a shorter favourable period, which reduces construction costs as a proportion of total production costs (Kikuzawa and Lechowicz 2011). When the favourable period is shorter, construction costs of deciduous leaves may be too high in relation to total plant production costs, which leads to an evergreen habit (Kikuzawa and Lechowicz 2011).

1.2.1 Shoot lifespan and associated growth parameters

In their review of leaf lifespan studies, Chabot and Hicks (1982) stated that, in light of resource economics, a plant should discard its leaves when the cost of maintaining them during the unfavourable period exceeds the benefit. The authors state that leaves should also be discarded when the cost of their loss is less than future benefits that would accrue from carbon and nutrient gain. As leaf construction becomes more energetically taxing, lifespan should increase to reduce overall costs (Chabot and Hicks 1982).

In a study of leaf longevity based on data in Wright et al. (2004) from 657 plant species from arctic tundra, tropical and boreal forests, deserts, and grasslands, there was a positive relationship between leaf construction cost and longevity, and a negative relationship between photosynthetic rate and leaf longevity (Kikuzawa et al. 2013). In grass species, adaptation to disturbance explained interspecific variation in leaf lifespan better than adaptation to site productivity (Ryser and Urbas 2000). This indicates that only when disturbance such as defoliation is minimal is there an advantage for plants to have greater leaf longevity, which in turn helps retain nutrients (Ryser and Urbas 2000).

Increased leaf longevity is associated with more efficient use of nutrients (Chabot and Hicks 1982; Escudero et al. 1992), as measured by photosynthate per milligram of nitrogen, which signals the ability of evergreen leaves to recover nutrients more efficiently from older leaves (Chabot and Hicks 1982). Leaf lifespan has been shown to decrease with decreasing growing season in deciduous plants, and increase in evergreen plants, with the prediction that extension of

growing season length would increase leaf turnover in evergreen plants, due to decreased leaf nitrogen content (Kudo et al. 1999). Given a warmer climate, it is possible that similar effects could be observed in roots.

Plant leaf traits are often collectively referred to as the Leaf Economics Spectrum (LES), which has been widely studied by many authors, including Chabot and Hicks (1982), Wright et al. (2004), Donovan et al. (2014), and Diaz et al. (2016). The LES refers to a specified set of multivariate correlations that constrain plant leaf traits to a single axis of variation (Osnas et al. 2013). Measures such as leaf mass per area (LMA), photosynthetic capacity, leaf nitrogen, leaf phosphorus, and dark respiration rate have been widely studied as metrics in the LES (e.g. Wright et al. 2004, Shipley et al. 2006, Poorter et al. 2014). Other plant traits encompassed by the LES have also been studied, such as plant leaf resource allocation (Wilson et al. 1999), longevity (Escudero et al. 1992, Kikuzawa et al. 2013, Kudo 1992), and evergreenness (Monk 1966).

Diaz et al. (2016) point to the balance between leaf construction costs and growth potential accounted for by the LES. There is also an economic trade-off between plant growth and defence potential, although species phylogeny has been reported to play a larger role than functional group (e.g., woody or deciduous; Villar et al. 2006). Noting the correspondence between the LES and the root, stem, and whole-plant economic spectra, Freschet et al. (2010) indicated that if leaf traits can be used to predict whole-plant traits, such as those that involve below-ground organs, this would be an asset in studying the ecological impacts of changes in vegetation. For example, these same authors have shown that in subarctic flora, lignin, carbon, and dry matter content, as

well as carbon to nitrogen ratios, have been shown to correlate between leaves and stems, leaves and roots, and stems and roots.

1.2.2 Shoot strategy: multi-season (evergreen) or single-season (deciduous)

For tree species, it is well known that the transition from southern to northern forest biomes is associated with a change in leaves from those that are annually renewed, typically deciduous broadleaves, to those that are evergreen, typically coniferous needles. Shortening of the growing season decreases the advantage of deciduous plants over evergreen ones (Givnish 2002). If the favourable period persists all year in a given area, plants can photosynthesize during this entire period, which explains why evergreen forests are found in tropical and sub-tropical regions, given sufficient precipitation (Kikuzawa and Lechowicz 2011). When the favourable period is shorter than the full year, it may be advantageous to shed leaves if maintenance costs would otherwise outweigh the benefits (Kikuzawa and Lechowicz 2011). For example, to maintain leaves during a cold period, leaves must invest in protection such as structural carbohydrates, which compromises their photosynthetic capacity during the favourable period (González-Zurdo et al. 2016).

When the unfavourable period is extremely short, it may be so difficult to offset the cost of leaf construction that an evergreen habit is most advantageous (Kikuzawa and Lechowicz 2011). That is to say, there may be insufficient time during a growing season in which to compensate for lost resources. In areas with a longer unfavorable period, evergreen plants have increased leaf

longevity and can persist for multiple years, in order to offset the leaf construction and maintenance costs and a shorter photosynthetic period (Kikuzawa and Lechowicz 2011).

Evergreens tend to photosynthesize at lower rates than deciduous species, but use nutrients more efficiently; they can thus achieve higher carbon gain at a given level of nutrient acquisition (Chabot and Hicks 1982). Evergreens benefit from increased nutrient retention time in leaves, which explains why they predominate at nutrient-poor sites (Escudero et al. 1992). In a raised sphagnum peat bog in eastern Ontario, bog evergreen species were shown to have higher photosynthate levels than bog deciduous species; levels were also higher for bog deciduous species than for than non-bog deciduous species (Small 1972). Kudo (1992) showed that, along a gradient of decreasing snow-free duration, evergreen shrubs (*Phyllodoce aleutica* and *Rhododendron aureum*) had extended leaf lifespans, whereas a deciduous shrub (*Sieversia pentapetala*) and herb (*Potentilla matsumurae*) had reduced leaf lifespans, adaptations thought to maintain a positive carbon budget in light of limited photosynthetic potential.

Given that high plant productivity requires adequate supply of nutrients and water, deciduous trees lose their advantage if these resources are in short supply (Schroeder 1998). When these conditions are present, and the growing season is short, coniferous trees predominate (Schroeder 1998). In addition, evergreen species with slower leaf turnover rates lose less carbon, reducing costs associated with producing new leaves (Chabot and Hicks 1982).

1.3 Root growth economics and phenology

Mommer and Weemstra (2012) have stated that, while resource allocation in roots is fundamentally different from that of leaves, there remains a dearth of study pertaining to belowground plant traits. Tissue structure and longevity of leaves has been found to be quite different from that of roots (Withington et al. 2006). In other studies, it was found that herbaceous monocots that possess winter-senescing leaves may also possess roots that survive the winter (Ryser and Kamminga 2009; Nieman, Hoogzaad, Marcotte, and Ryser, unpublished results).

In order to understand whole-plant resource allocation strategy, it is necessary to study root production across multiple ecosystems (Sloan et al. 2016). To date, the study of correlations between root and leaf traits has produced mixed results. Tjoelker et al. (2005) found a correlation between leaf and root longevity in grassland and savannah species, but not between specific root length (SRL; ratio of root length to dry mass) and specific leaf area (SLA; ratio of leaf area to dry mass). Among temperate trees, Withington et al. (2006) found no correlation between leaf and root longevity, but did find a positive correlation between SRL and SLA. Correlations between SRL and SLA were found by Wright and Westoby (1999) in woody dicot seedlings and by Freschet et al. (2010) in woody and herbaceous subarctic flora. Ryser (1996) found a relationship between root and leaf tissue density in five grass species, but with variation in the direction of the relationship among the species.

While the LES has been widely investigated, not nearly as much has been written about the concept of a Root Economics Spectrum (Mommer and Weemstra 2012; Roumet et al. 2016) or a Plant Economics Spectrum (Freschet et al. 2010, Reich 2014). However, the broader categories of root traits (Jackson and Caldwell 1989, Jackson et al. 1997) and whole plant traits (Donovan et al. 2014, Edwards et al. 2014) have received wider study. Root traits have been linked to the LES due to the fact that fine roots are analogous to leaves—both tissues experience rapid turnover and play an important role in resource uptake (Donovan et al. 2014).

In their review of root phenology studies, Radville et al. (2016) state that plant phenology is a key indicator of climate change response, and that this response can profoundly influence net primary production, species composition, greenhouse gas budgets, and other ecological processes. Given that nutrients may be the most limiting growth factor for wetland graminoids such as sedges, there is continuous resource allocation to roots, as opposed to early allocation to shoots as in woody vegetation (Sloan et al. 2016).

In an experiment with root growth at varying nutrient supply, two species of grass (*Dactylis glomerata* and *Brachypodium pinnatum*) responded to increasing nitrogen and phosphorus by decreasing their root length per unit of plant dry mass (root length ratio; RLR) and increasing their leaf area per unit of plant dry mass (leaf area ratio; LAR; Ryser and Lambers 1995). In a field experiment (Schläpfer and Ryser 1996) and in a pot experiment (Ryser 1996), it was shown that grass species that were better adapted to exploiting nutrients initially grew faster even under conditions of limited nutrients, but that the species adapted to low nutrient availability by having longer nutrient residence periods produced greater biomass in the long term. Grass species with

low root and leaf tissue mass density have been shown to produce a large root network and leaf area and to be characteristic of fast-growing species from nutrient-rich habitats (Wahl and Ryser 2000). Caldwell et al. (1985) showed the importance of root length, mycorrhizal fungi, and water uptake potential in nutrient competition. In their review of root phenology studies, Radville et al. (2016) stated that certain plant species may gain a competitive advantage by faster initiation of root growth to uptake limited resources, such as water.

In a study of the influence of temperature and water potential on root growth of white oak in a temperate forest in Missouri, Teskey and Hinckley (1981) found that root production continued after leaf senescence, halting in September, resuming in October, halting in January, and resuming in February until peak elongation rate in April.

In a study of the synchrony of root and leaf phenology in sub-Arctic plant communities, root production began early in the growing season in sedges, following peak leaf production, and there was a significant relationship between soil temperature and rate of root production (Sloan et al. 2016). In woody plants, there were inconsistent relationships between temperature and root production, which signals differences between plant functional types and highlights the need for separate study of leaf and root production dynamics in carbon and nutrient models, in order to understand the effects of vegetation change due to climate change (Sloan et al. 2016). In addition, given that root carbon inputs are an important driver of methane production, differences in the timing of carbon inputs may influence the timing and magnitude of emissions (Sloan et al. 2016).

Wetland soils are known for their potential to be slow in adjusting temperature in response to changes in solar irradiation levels (Kadlec 1999). Changes in the temperature of wetland soils have been shown to co-occur with hydrological changes such as in water chemistry (Waddington and Price 2000), which is thought to drive the relationship between vegetation and wetland water levels (Pollock et al. 1998).

1.3.1 Root lifespan and associated growth parameters

Roots experience turnover for many reasons, one of which is attributed to decreased nutrient uptake as they age (Eissenstat and Yanai 1997). In temperate trees, the ratio of root nitrogen to carbon was negatively correlated with root longevity (Withington et al. 2006), indicating that high productivity and fast growth is negatively associated with lifespan, in a similar manner to leaves (Wright et al. 2004). When grown under the same conditions, fast-growing grass species originally from sites with high nutrients had shorter leaf and root lifespan than those originally from sites with lower nutrients, and root and leaf tissue density was negatively correlated with root and leaf turnover (Ryser 1996). This indicates a trade-off between nutrient acquisition and resource conservation among grass species (Ryser 1996). Another study confirmed the variation in root parameters that reflect trade-offs between resource acquisition (e.g. high SRL, low diameter, low root dry matter content or RDMC) and resource conservation (e.g. low SRL, high diameter, high RDMC), which signals similar trade-offs at the plant community level (Prieto et al. 2015).

Decreased temperature is another parameter that has been associated with increased root longevity in previous studies. In a study of fine root mortality in two sugar maple forests, roots in the more northerly forest were shown to have greater lifespan, and warmer soil temperatures were associated with faster root death at the southern site (Hendrick and Pregitzer 1993). However, in an experiment that tested the effects of day length and temperature on root length in tundra graminoids, temperature was not directly correlated with root growth during the growing season, despite the presence of near-freezing soil temperatures (Shaver and Billings 1977). Other studies have shown that root accumulation is a function of change in growing season length and not of soil temperature (Fitter et al. 1998).

In a study of root production and turnover of graminoids from a wet tundra ecosystem near Barrow, Alaska, there was a strong positive correlation between new root production and the age of tillers, annual root turnover in the ecosystem was estimated to be approximately 25% of the biomass of live roots, and root lifespan varied from a single season to multi-season roots that persisted for as long as eight years in some cases (Shaver and Billings 1975).

Roumet et al. (2006) studied differences in root traits between perennial plants (overwintering vegetative structures) and annual plants (seeds as the only overwintering structures) grown in the field. It should be noted that the terms perennial and annual did not refer to the use of a multi-season or single-season root growth strategy of perennial plants as in the present study. The authors found that roots of annual species demonstrated enhanced resource acquisition potential due to their low-density roots with high nitrogen concentration, while roots of perennial species showed greater persistence due to greater thickness and density. It is thought that root traits

parallel leaf traits, with the roots of annual species maximizing below-ground resource acquisition, and the roots of perennial species conserving below-ground resources (Roumet et al. 2006). The authors determined that the high SRL (thin, low density roots) of annuals might allow for maximization of root surface area in order to efficiently exploit resources, grow rapidly, and quickly complete the life cycle, whereas the low SRL (thick, high density roots) of perennials was thought to be a survival adaptation to highly competitive habitats (Roumet et al. 2006). Species with a slow growth are often found in stressed habitats, whereas species with rapid growth are often found in productive environments (Poorter et al. 2014). Wright and Westoby (1999) demonstrated the high relative growth rate of woody dicots that maximize leaf and root absorptive surface.

1.3.2 Root strategy: multi-season (evergreen) or single-season (deciduous)

Root lifespan is commonly considered a continuous variable, ranging from months (Fitter et al. 1998) to years (Shaver and Billings 1975). In their review of root phenology studies, Radville et al. (2016) suggested that roots and shoots do not demonstrate the same patterns of winter dormancy, with 89% of studies on winter phenology finding root growth during this season.

There are indications that root lifespan in temperate wetlands can be classified as using either a multi-season or single-season growth strategy (Ryser and Kamminga 2009; Nieman, Hoogzaad, Marcotte, and Ryser, unpublished results). In a root survival study of wetland graminoids, the majority of axile roots and the few lateral roots found survived until mid-December in *Carex oligosperma, Carex stricta, Phalaris arundinacea* and *Scirpus microcarpus*; axile roots of

Dulichium arundinaceum survived, but its lateral roots died in October; and all roots of *Sparganium androcladum* died in October, which shows that the timing of leaf mortality does not coincide with the timing of fine root mortality (Ryser and Kamminga 2009).

Local variation in soil temperature influences the occurrence of these two contrasting strategies, as shown in a study of soil temperature and wetland monocots that found that species with single-season roots could not be found at sites with the shortest growing season and coolest soil temperatures (Gagnon et al. 2015).

1.4 Study overview

The present study focused on root growth strategies of wetland monocots in northern Ontario, Canada. Annually renewed roots are referred to as single-season, and overwintering roots are referred to as multi-season. The primary research question investigated whether wetland plant species with multi-season roots (sMSR) and species with single-season roots (sSSR) respond to growing season length in a similar manner as species with evergreen leaves and species with deciduous leaves, respectively. The objective of this study was to determine to what extent root and shoot phenology of species with contrasting root growth strategies (i.e., multi- or single-season) are related to soil temperature, across a climatic gradient of growing season length.

The hypothesis was, when the growing season is short, a multi-season root growth strategy is more advantageous than a single-season root growth strategy, which implies that the production of new roots in spring delays plant growth. The predictions were: (1) sSSR would experience delayed shoot growth early in the growing season as compared to sMSR, with the delay

attributed to the production of new roots prior to shoot production; and (2) sMSR would invest in late-season root production to a greater degree than sSSR.

A full accounting of the costs to plants of over-wintering roots is not yet known, but the production of multi-season roots reduces the energetic losses associated with the production of new roots. If the growing season is too short, it would not be economical to grow new roots every year, as the energetic benefits of doing so would not outweigh the energetic costs. In addition, multi-season roots enable resource acquisition during marginally productive periods of the growing season, such as early spring.

All the chosen study species are classified as perennial in the literature because they possess overwintering vegetative structures such as rhizomes, bulbs, buds or tubers (USDA NRCS 2016, Newmaster et al. 1997). In Roumet et al. (2006), roots of annual species employed a resource acquisitive strategy, while roots of perennial species employed a resource conservative strategy, in parallel with leaf traits. This definition of annual and perennial plants does not account for the multi- or single-season nature of roots. By definition, annual species produce annual roots, but perennial species may produce single-season or multi-season roots. The present research constitutes the first time that root and shoot phenology of wetland plant species with contrasting root growth strategies were studied in the field across a gradient of growing season length. Apart from Ryser and Kamminga (2009); Nieman, Hoogzaad, Marcotte, and Ryser (unpublished results); and the present research, roots of perennial plants have not been studied in the context of the multi- or single-season nature of root growth.

In order to understand the effect of growing season length on the root growth strategy of the study species, study sites were chosen along a north-south climatic gradient. At the northern end of the study area, study sites were chosen in the northern and southern zones of Lake Superior Provincial Park, due to the transition between the boreal forest in the north end of the park to the Great Lakes–St. Lawrence forest in the south end of the park. At the southern end of the study area, study sites were chosen on Manitoulin Island. The annual average temperature in Wawa, approximately 15 km north of the northern border of Lake Superior Provincial Park, is 1.7 °C, while in Gore Bay, located in central Manitoulin Island, it is 5.2º C (Environment Canada 2016).

The study was carried out by installing root ingrowth cores in wetland soil substrate and subsequently measuring root length that grew therein over the course of the study period, from fall 2013 to spring 2015. Shoot growth during that period was measured as well.

1.5 Study significance

This study is unique in that it: (1) Investigated root and shoot growth across a climatic gradient in a field setting, specifically in wetland ecosystems. (2) Assessed the ecological significance of contrasting root growth strategies of monocot species, rather than viewing root lifespan as a continuous variable, and is the first to do so in the field. There is a need for further study of interspecific variation in root longevity, senescence, and decomposition in response to growing season length (Eissenstat and Yanai 1997, Scheffer and Aerts 2000, Ryser and Kamminga 2009). (3) Studied both above-ground and below-ground plant material over the growing season, which was important due to widespread acknowledgement of the need for further knowledge of root

phenology in order to enhance our understanding of carbon cycling (Abramoff and Finzi 2015, Blume-Werry et al. 2016, Radville et al. 2016).

Chapter 2: Methods

2.1 Study species

For this investigation, three species with multi-season roots (sMSR) and three species with single-season roots (sSSR) were selected. All study species are wetland monocots (5 graminoid and 1 forb) relatively common in northern Ontario, with a wide distribution. As mentioned previously, all the study species are classified as perennial in the literature (USDA NRCS 2016, Newmaster et al. 1997) because they possess overwintering vegetative structures. Determination of the root growth strategy of these species is based on Ryser and Kamminga (2009) and unpublished results by Tim Nieman, Yvonne Hoogzaad, Elize Marcotte, and Peter Ryser. These investigations determined the root growth strategy of study species through live root measurements using 2,3,5-triphenyltetrazolium chloride (TTC; method described in section 2.4.1) with garden-grown plants in which overwintering pots were prevented from freezing. Root vitality was assessed late in winter as well as early in spring.

Among the study species, *Carex lasiocarpa, Eleocharis palustris,* and *Trichophorum cespitosum* possess multi-season roots, while *Rhynchospora fusca, Sagittaria latifolia,* and *Sparganium americanum/emersum* have single-season roots. Table 2.1 provides an overview of the preferred habitat of the study species. Appendix 1 provides additional life history information pertaining to study species.

Table 2.1. The six monocotyledonous wetland study species with contrasting root strategies. Information on preferred habitat is based on Newmaster et al. (1997).

Species	Common name	Family	Growth form	Habitat
Species with multi-season roots				
Carex lasiocarpa Ehrh.	Wire sedge	*Cyperaceae* (Sedge)	Graminoid	Fens, peat marshes
Eleocharis palustris (L.) Roem. & Schult.	Marsh spike-rush	*Cyperaceae* (Sedge)	Graminoid	Marshes, riverbanks, lakeshores, wet fens
Trichophorum cespitosum (L.) Hartm.	Tufted clubrush	*Cyperaceae* (Sedge)	Graminoid	Rich fens, Lake Superior shores
Species with single-season roots				
Rhynchospora fusca (L.) W.T. Aiton	Brown beakrush	*Cyperaceae* (Sedge)	Graminoid	Open fens
Sparganium americanum[1] Nutt.	American bur-reed	*Sparganiaceae* (Burreed)	Graminoid	Shallow water of marshes, rivers, ponds
Sparganium emersum Rehmann	Common bur-reed	*Sparganiaceae* (Burreed)	Graminoid	Shallow water of marshes, rivers, ponds
Sagittaria latifolia Willd.	Arrowhead	*Alismataceae* (Water plantain)	Forb	Marshes, lakes, ponds, streams, wet ditches

[1]*S. americanum* is closely related and ecologically similar to *S. emersum*, thus they are treated as one species.

2.2 Study sites

The study region represents a climatic gradient ranging from an annual average temperature of 1.7 ºC with a growing season of as little as 130 days to an annual average temperature of 5.2 ºC with a growing season of up to 170 days. The study sites were located in two regions—Lake Superior Provincial Park, south of Wawa, Ontario, and Manitoulin Island, near the north shore of Lake Huron (Table 2.2). Lake Superior Provincial Park was further divided into northern and southern zones; taken together with Manitoulin Island, there were three study zones in total. The distance from the north end of Lake Superior Provincial Park to study sites at the west end of Manitoulin Island is approximately 270 km, and to study sites at the east end of Manitoulin Island is approximately 315 kilometres. The annual average temperature in Wawa in the northern end of the study region is 1.7 °C, and is 5.2 ºC in Gore Bay in central Manitoulin Island in the southern end of the study region (Environment Canada 2016; Figure 2.1). Maps of study sites were created using ArcGIS Online (Esri, Province of Ontario 2016).

Within the boundaries of Lake Superior Provincial Park, there is an ecotone, or transition zone, between the boreal and Great Lakes–St. Lawrence forest regions of the temperate forest biome (Ontario Ministry of Natural Resources and Forestry 2016). The Great Lakes–St. Lawrence Forest occupies the majority of the park landmass, while approximately one tenth is occupied by the boreal forest in its extreme northern end (White 1988). Between these two regions in the park, a mixed forest is present along a northerly portion of the Lake Superior coast, in the major river valleys, and around the larger lakes of the southern and central areas (White 1988; Figure 2.2). This forest transition zone provides an excellent opportunity to investigate plant behaviour

across this border. Accordingly, study sites in Lake Superior Provincial Park were chosen in both northern and southern zones (Figure 2.3).

The climatic gradient within the study area is also associated with a gradient of growing season length, which is temperature-dependent. The growing season in Lake Superior Provincial Park is approximately 130 to 140 days in the northern zone and 140 to 150 days in the southern zone, as compared to 160 to 170 days at the study sites on Manitoulin Island in the southern region of the study area (Agriculture and Agri-food Canada 2014; Figure 2.4). Soil and aerial temperature factor prominently in determining the actual growing season length at individual sites.

The species composition of the forests in the study regions are representative of the climate. The northern part of the Algoma region in which Lake Superior Provincial Park is located has forest dominated by white spruce (*Picea glauca*), black spruce (*P. mariana*), balsam fir (*Abies balsamea*), jack pine (*Pinus banksiana*), white cedar (*Thuja occidentalis*), tamarack (*Larix laricina*), white birch (*Betula papyrifera*), red maple (*Acer rubrum*), and speckled alder (*Alnus rugosa*; Rowe 1972). White pine (*P. strobus*) and red pine (*P. resinosa*) are common as well (White 1988). Sugar maple (*A. saccharum*) and yellow birch (*B. alleghaniensis*) dominate forests in the southern part of the park, while black spruce, balsam fir, and white birch dominate the forests in the northern part (White 1988).

Manitoulin Island's forest is dominated by sugar and red maple, white elm (*Ulmus americana*), basswood (*Tilia americana*), yellow birch, red oak (*Quercus rubra*) and bur oak (*Q. macrocarpa*), hop-hornbeam (*Ostrya virginiana*), beech (*Fagus grandifolia*), largetooth aspen (*Populus grandidentata*), white birch, white ash (*Fraxinus americana*), black ash (*F. nigra*), and

red ash (*F. pennsylvanica*), with red and white pine, white spruce, balsam fir, hemlock (*Tsuga canadensis*), and red cedar (*Juniperus virginina*) present in smaller numbers (Rowe 1972). In Appendix 2, Figures A1 to A9 illustrate the numbered study sites and species under investigation at a higher resolution. While the majority of study species are present at sites in the northern and southern zones at Superior, as well as at Manitoulin, there are exceptions. A *T. cespitosum* study site was not found within Superior's northern zone, an *S. latifolia* site was not found within Superior's southern zone, and an *R. fusca* site was not found at Manitoulin.

Table 2.2. Geographic information pertaining to study sites.

Study region	Site number	Species	Geographic coordinates
Lake Superior Provincial Park northern zone[1]	1	*Sparganium americanum*	N47° 44.753' W84° 50.336'
	2	*Sagittaria latifolia*	N47° 45.052' W84° 49.921'
	3	*Eleocharis palustris*	N47° 45.023' W84° 49.578'
	4	*Carex lasiocarpa*	N47° 42.894' W84° 48.742'
	5	*Rhynchospora fusca*	N47° 42.166' W84° 43.846'
Lake Superior Provincial Park southern zone[2]	6	*Sparganium americanum*	N47° 32.574' W84° 48.213'
	7	*Trichophorum cespitosum*	N47° 27.974' W84° 47.334'
	8	*Carex lasiocarpa*	N47° 16.971' W84° 33.014'
	9	*Eleocharis palustris*	N47° 16.909' W84° 32.657'
	10	*Rhynchospora fusca*	N47° 16.909' W84° 32.655'
Manitoulin Island[3]	11	*Carex lasiocarpa*	N45° 47.635' W82° 44.940'
	12	*Trichophorum cespitosum*	N45° 47.880' W82° 44.798'
	13	*Sparganium emersum*	N45° 36.402' W82° 06.155'
	14	*Eleocharis palustris*	N45° 40.543' W82° 02.380'
	15	*Sagittaria latifolia*	N45° 40.703' W82° 02.294'

Growing season length: [1] 130 – 140 days, [2] 140 – 150 days, [3] 160 – 170 days.

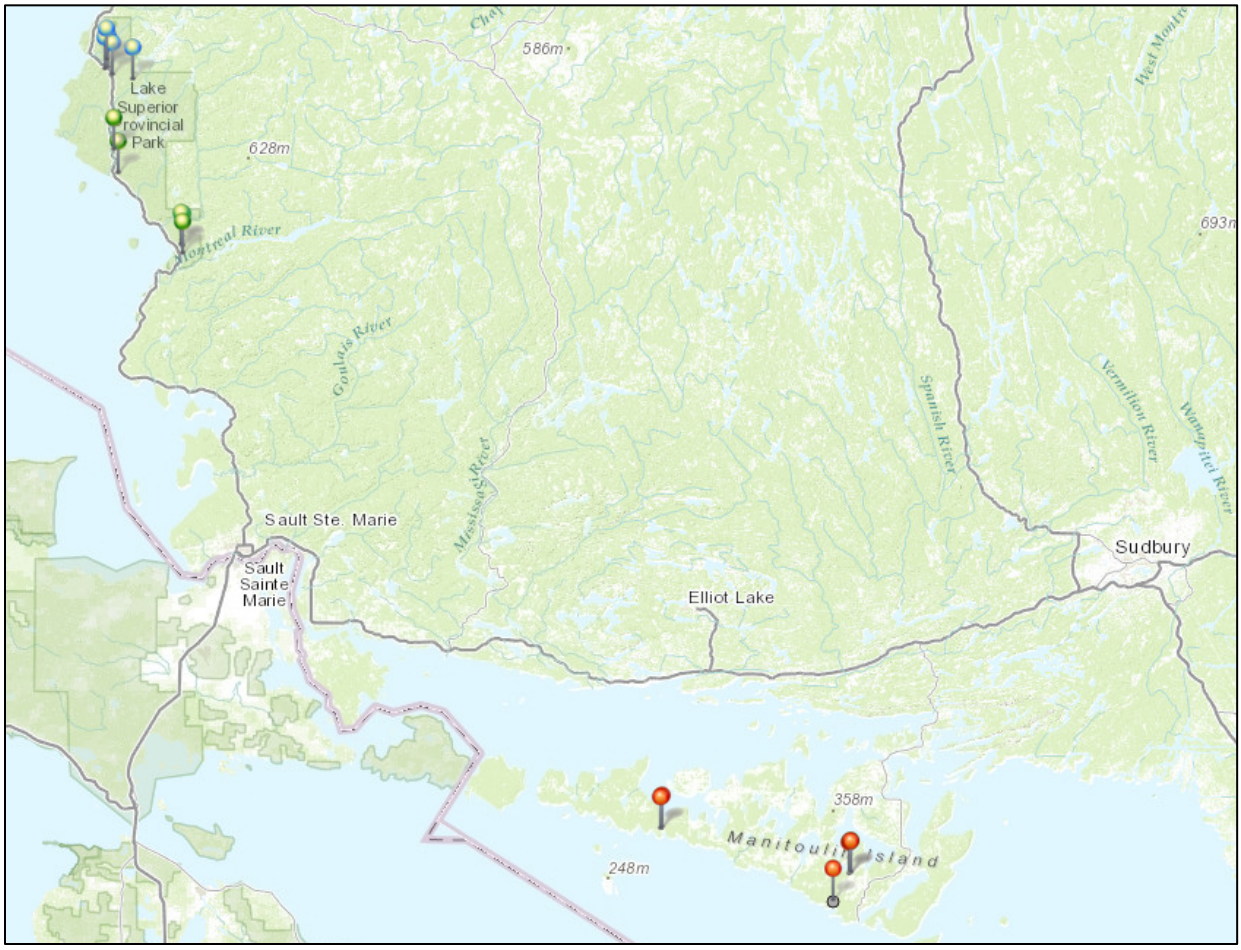

Figure 2.1. Map of study area.

Lake Superior Provincial Park sites in northern zone (blue markers) and southern zone (green markers), and Manitoulin Island sites (red markers).

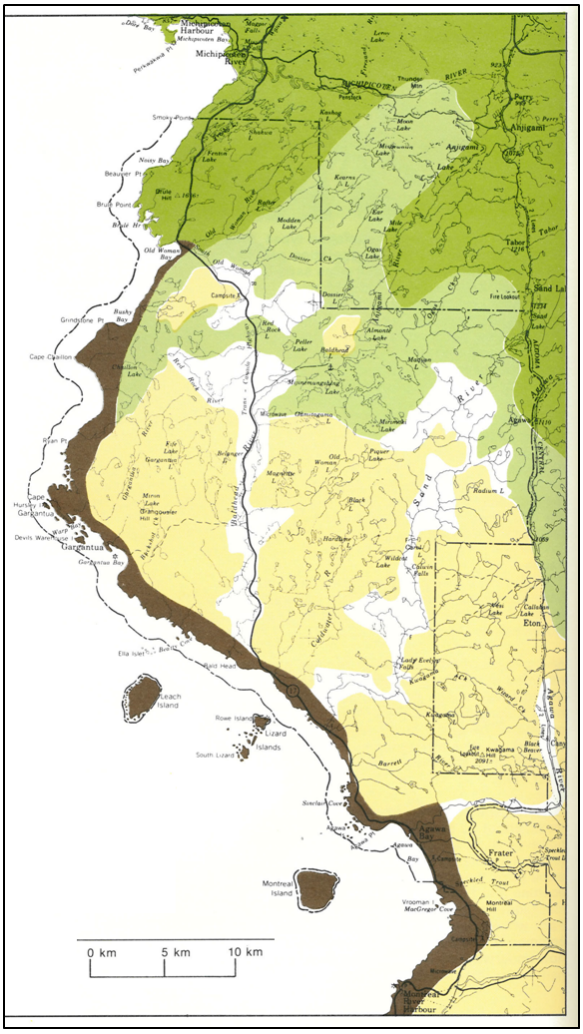

Figure 2.2. Forest transition zone in Lake Superior Provincial Park.

Transition zone (light green) shown between boreal (dark green) and Great Lakes – St. Lawrence (tan) forest regions. Coastal zone indicated in dark brown (Ontario Ministry of Natural Resources 1979).

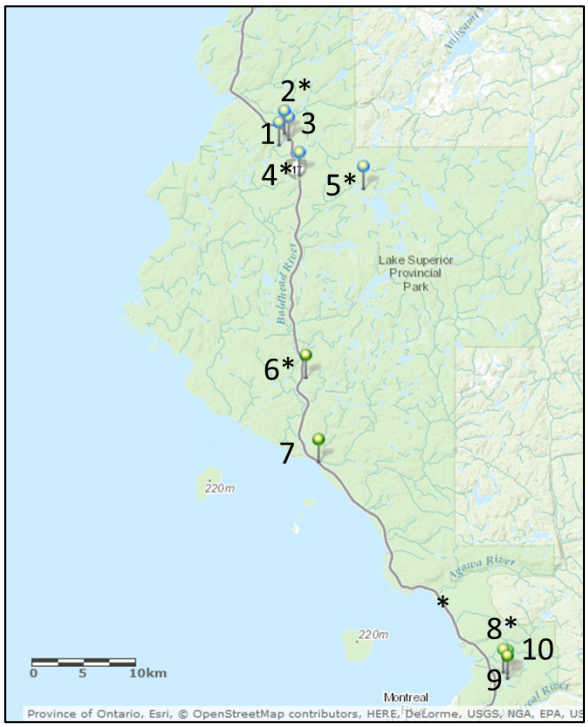

Figure 2.3. Lake Superior Provincial Park study sites.

Northern zone: sites 1-5 (blue markers), southern zone: sites 6-10 (green markers). Species at sites: *S. americanum* (1, 6), *S. latifolia* (2), *E. palustris* (3, 9), *C. lasiocarpa* (4, 8), *R. fusca* (5, 10), *T. cespitosum* (7). Aerial datalogger sites denoted by asterisk (*); see section 2.3.2.

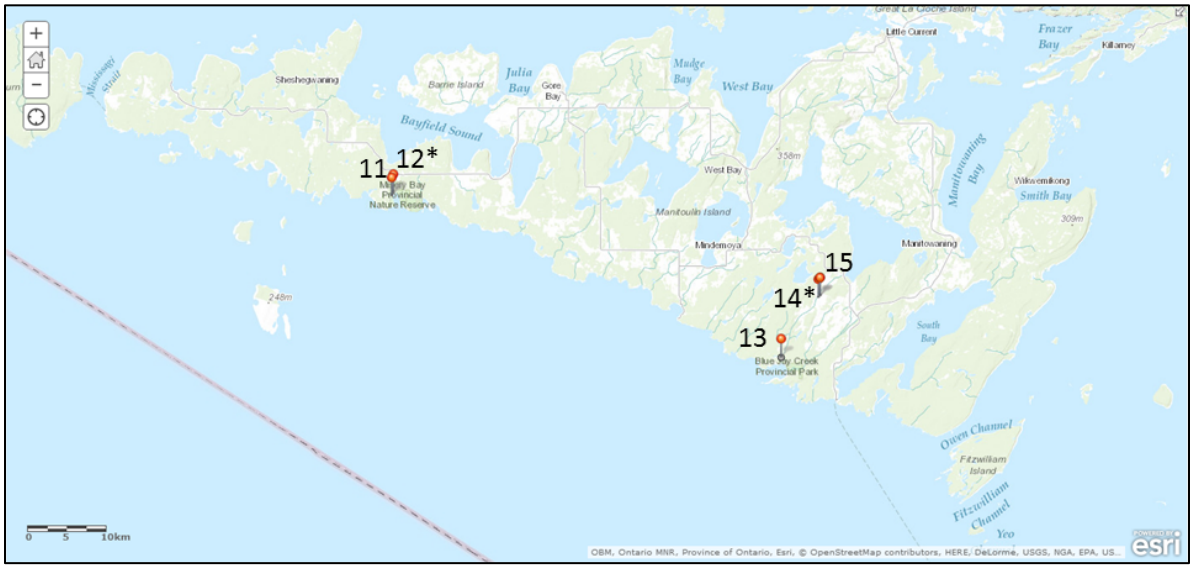

Figure 2.4. Manitoulin Island study sites.

Species at sites: *C. lasiocarpa* (11), *T. cespitosum* (12), *S. emersum* (13), *E. palustris* (14), *S. latifolia* (15). Aerial datalogger sites denoted by asterisk (*); see section 2.3.2.

2.3 Experimental design

2.3.1 Root growth

Cylindrical root ingrowth cores were installed in wetland substrate at each study site in order to capture root growth within them over the course of the study, from the fall of 2013 to the spring of 2015. The cores were made of 0.6 mm steel mesh, and were 4 cm in diameter, 27 cm in length, and 340 cm^3 in maximum volume (Gerard Daniel Worldwide Canada, Mississauga, Ontario).

The cores were installed within uniform stands of the study species. Holes of approximately 5 cm diameter were dug under water into the soil, with the use of a soil auger (Eijkelkamp Soil &

Water, Giesbeek, Netherlands). The cores were filled with local substrate after sieving with a 1 cm grid mesh to remove previously grown roots. Local substrate was used to replicate local soil conditions and to minimize plant response to substrate heterogeneity, which has been reported by Crick and Grime (1987) and Wijesinghe and Hutchings (1997) to occur in certain herbaceous species. Despite sieving of soil used to fill the cores, some previously-grown roots that were too small to be removed during the sieving process were likely still present in the local substrate used to fill the cores.

For *T. cespitosum*, the cores were filled with sieved and autoclaved horticultural peat, which best corresponds with the peat in which this species naturally occurs. The soil was autoclaved in order to avoid introduction of foreign organisms, in compliance with permitting requirements for conducting research in provincial parks, mandated by the Ministry of Natural Resources and Forestry. The soil-filled cores were then inserted into the substrate. A total of 200 cores were installed at 10 sites in Lake Superior Provincial Park, hereafter referred to as Superior, and 100 cores were installed at 5 sites on Manitoulin Island, hereafter referred to as Manitoulin. *T. cespitosum* at Superior proved to be a difficult site at which to install cores, due to site characteristics. This species grew in shallow tussocks of peat formed on bare rock along the Lake Superior shoreline and some of the cores could not be inserted to their full length, with the consequence that cores were not completely filled with soil substrate.

At each site, 4 replicate posts were installed, with each post in the center of a ring of 5 cores. String was tied from the post to each core to facilitate locating cores (Figure 2.5).

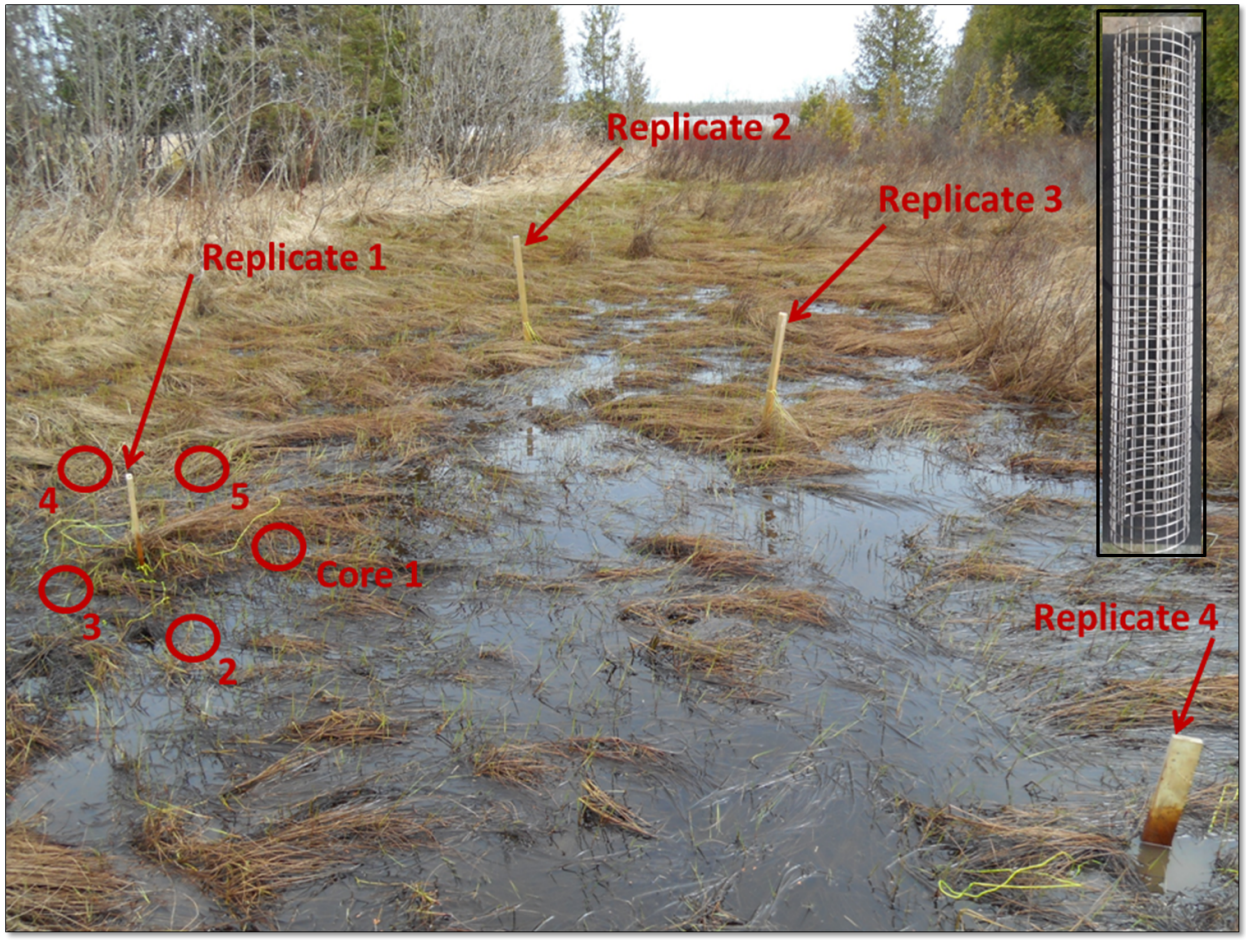

Figure 2.5. Field setup of posts and root ingrowth cores, with core featured in inset at top right.

2.3.2 Soil and air temperature

To monitor soil and air temperature, iButton® temperature data loggers (DS1921G-F5# Maxim Integrated, San Jose, USA) with a resolution of 0.5 ºC, were programmed to measure temperature every 255 minutes, placed in waterproof polyvinyl chloride containers, and installed at field sites. Four dataloggers were installed in the wetland substrate at each site; 2 at a depth of 20 cm, and 2 at a depth of 10 cm. Six sites throughout Superior and 2 throughout Manitoulin were chosen to install aerial temperature dataloggers (Figures 2.3, 2.4). They were mounted on

trees at a height of approximately 1.5 metres in housing units to protect them from direct solar radiation, while open spaces ensured unrestricted airflow. In total, 60 soil temperature dataloggers and 16 air temperature dataloggers were installed in fall 2013. They were replaced by new dataloggers in summer 2014 to ensure data recovery.

2.3.3 Soil parameters

Additionally, soil pH and redox potential were measured to describe the sites in further detail, but were not statistically analysed. Soil samples were collected from the top 5 cm of soil at all sites at Harvest 1. Soil samples were also collected below 5 cm if the bottom layer was visually distinct from top layer. At the lab, pH was measured (Milwaukee® portable pH/EC/TDS, model SM802) after mixing 1 part soil with 2 parts distilled water. As waterlogged plants have been shown to experience root and shoot growth constraints due to anoxic substrate conditions (Ryser et al. 2011), reduction-oxidation potential was measured with an Accumet platinum combination electrode with AgCl as reference (Fisher Scientific, Pittsburgh, PA, USA) at Harvests 2 and 3 by collecting soil, mixing one part soil with one part water from the site location, and taking a reading from a redox probe that was left in the sample for a minimum of 30 minutes. Results were not statistically analyzed and are contained in Appendix 3.

2.4 Data collection protocols

2.4.1 Root growth

Twenty cores were installed at each field study site to provide 4 replicate samples for each of the 5 harvests, which were carried out between spring 2014 and spring 2015. At harvests, cores were removed from the wetland substrate by cutting the soil with a serrated knife as close to the perimeter of the cylinder as possible. It should be noted that at the time of harvest, at certain sites, soil at the top of some ingrowth cores was missing. This was due to a drop in water levels at certain sites after flooding, which washed soil out from the top few centimetres of the cores, or the shallowness of soil profiles at other sites. However, missing soil could not be quantified in all cores. At sites with saturated soils, cores had to be removed from the substrate and bagged instantaneously to prevent the contents of cores from spilling out.

Cores were bagged and kept cool with ice packs to reduce root metabolism during transportation, until further processing. Cores were kept refrigerated until processing their contents. Substrate from the cores was placed in plastic containers, filled with water, and sieved repeatedly with successively finer mesh (2 mm grid, followed by 1 mm) to remove the soil and separate and wash the roots. After careful visual assessment or with a microscope when necessary, roots that were determined to be from non-target species were removed from the sample. Roots were then cut into approximately 1 cm sections with a razor blade, to encourage subsequent absorption of a 2,3,5-triphenyltetrazolium chloride (TTC) solution, described below.

Every effort was made to prevent loss of root fragments at each stage of the root collection process. To prevent root metabolism and mortality prior to staining with TTC, harvests were completed as quickly as possible, with separate Superior and Manitoulin harvests performed in succession, as rapidly as possible. However, given the number of samples, the considerable physical distances between study sites, and the necessity to process roots as quickly as possible, this increased the duration of individual harvests. Harvests at Manitoulin were generally completed within two days and transported to the lab at Laurentian University for processing. As there were twice the number of study sites at Superior compared to Manitoulin, and given the distance of the Superior sites from Laurentian University, samples were processed in a makeshift laboratory at Lake Superior Provincial Park headquarters at Red Rock Lake. The delay between core harvest and core processing ranged from several hours to several days. Appendix 4 provides sample size data of roots and shoots, for individual sites and species and across harvests, regions, and strategies, as well as species codes.

Date ranges of harvests are as follows. Harvest 1: May 8-July 5, 2014 at Manitoulin (days 128-186; flooded conditions at one site until July 5, 2014), June 10-June 25 at Superior (days 161-176; late spring thaw); Harvest 2: July 17-18 at Manitoulin (days 198-199), July 28-August 6 at Superior (days 209-218); Harvest 3: September 3-12 at Superior (days 246-255), September 20 at Manitoulin (day 263); Harvest 4: October 9-17 at Superior (days 282-290), October 27 at Manitoulin (day 300); Harvest 5: May 2-3, 2015 at Manitoulin (days 122-123), May 7-17 at Superior (days 127-137).

Vitality of roots that grew into the cores was assessed by staining with TTC, as practiced by Ryser and Kamminga (2009). Roots were placed in 25 ml glass vials and filled with a solution of

0.3% TTC and 10 mM glucose. Across all species and sites, the median root length measured at Harvest 1 was 351 cm, while median root length at Harvest 5 was 1474; across all harvests, species, and sites, the minimum root length was 23 cm and the maximum root length was 6371 cm.

As TTC is light-sensitive, the vials were wrapped in tinfoil to prevent exposure to light, and incubated at 30 ºC for a minimum of 24 hours. After incubation in the TTC solution, live root tissue was indicated by a chemical reaction in which TTC is reduced to formazan, which is pigmented red, by dehydrogenase enzymes (Comas et al. 2000). The glucose in the solution aided in coloration in the event that root respiration was substrate limited (Ryser and Kamminga 2009).

It was important to process root samples from cores as quickly as possible, to prevent root mortality before measurement. Thus, roots could not always be removed from the TTC solution and placed in ethanol after exactly 24 hours, as field and lab work was conducted alternately until each harvest was complete. Some vials remained in TTC solution for up to 72 hours. To quantify the percentage of root tissue exposed to TTC and reduced to formazan, samples were selected randomly from each vial of roots, in sufficient number to ensure a minimum of 45 to 50 root counts per sample. Roots were observed under a dissecting microscope (Leica EZ4, Leica Microsystems Switzerland Ltd.). The percentage of live root length was determined by counting the number of red-stained root fragments in the total. If the coefficient of variation (CV) among the three samples was greater than 30%, additional root samples were selected and measured until the CV was below 30%, or to a maximum of 6 samples.

To measure the total root length in each core, the modified line intersect method and formula was used, as described by Newman (1966) and modified by Tennant (1975). Harvest 2 was the first harvest to be measured, and an attempt was made to separate living roots from dead roots by visual assessment, by discarding roots that were degraded, limp, or frayed. This proved to be an unreliable method and was not used in the measurement of other harvests. Only extremely decomposed root fragments were discarded during root length measurement for all other harvests, as they were thought to have grown prior to core installation and not been successfully sieved from the local substrate used to fill the cores. Data from Harvest 2 was not statistically analysed, but results from this harvest should be treated with caution, regardless.

2.4.2 Shoot growth

To estimate above-ground growth, samples of shoots (stems, leaves, and flowers) that were representative of average growth were collected within a few metres of each study site, as determined by visual assessment. During each harvest, approximately 10 shoot samples were collected from each species at each site. They were kept cool using ice packs during transportation to the lab. Dry mass was determined after drying at 70 to 75 °C for 48 hours.

2.5 Calculations and statistical analysis

2.5.1 Soil and air temperature

The metric used to measure growing season length was Growing Degree Days (GDD), calculated using temperature measurements taken by dataloggers at 255-minute intervals at each site. Each GDD temperature measurement interval represents the product of the length of that interval and

the temperature difference from the threshold temperature, for positive temperature values only. GDD, then, represents the temperature sum within specified date ranges for which the temperature was above a chosen threshold. In the present study, a temperature threshold of 5 ºC was selected because root growth has been reported to occur near 0 °C, but is minimal below 6°C (Kummerow and Ellis 1984, Alvarez-Uria and Körner 2007). In their review of root phenology studies, Radville et al. (2016) confirmed the limitation of root growth below 5 °C in temperate environments and the lack of winter root growth seen across many ecosystems in seasonal environments. Selecting a threshold of 5 ºC for growing degree day assessment has been practiced by Bennie et al. (2010) and noted in Radville et al. (2016). To characterize the overall temperature of a study site, temperature data from all 4 dataloggers installed at each site was averaged. However, in some cases 1 or 2 dataloggers were missing or malfunctioned, resulting in data loss.

To provide an overview of temperature differences between study sites, GDD totals were calculated for January to December 2014. Additionally, GDD for five time periods in 2014 were calculated: first winter (January to March), spring (April to June), summer (July and August), fall (September and October), and second winter (November and December). Differences among study zones (Superior north, Superior south, Manitoulin) during the growing season were tested with an ANOVA of GDD for the year 2014, and a repeated measures ANOVA of GDD in spring, summer and fall. Adding data from November and December 2013 did not change results and are not shown. Dependent variables in the models were soil and air temperature sum (GDD) for 2014 and for the growing season (April – October); independent variables were zone, season,

and strategy (multi- vs. single-season root growth); and independent interaction variables were zone × season and strategy × season.

2.5.2 Root and shoot growth

For root length and shoot dry mass, the median value from all replicates at a given site and harvest was used (see Appendix 4 for sample sizes), in order to reduce the effect of high variation of ecological field data. Due to the large distances between the sites and the considerable field work required to obtain samples at each harvest, the date of a given harvest varied by several weeks among the sites. As seasonal changes in root and shoot growth can be rapid, this increases error variation in the data. To correct for this, median variable values were calculated at average dates in 2014 between two consecutive harvests: average day for Harvests 1 and 2 (day 183, July 2), average day for Harvests 2 and 3 (day 230, August 18), and average day for Harvests 3 and 4 (day 273, September 30). Root length (RL) and shoot dry mass (SM) at average harvest dates were calculated assuming a linear change from one harvest to the next, using the following formula:

$$
\begin{aligned}
&RL\ or\ SM\ at\ average\ day\ of\ harvest \\
&\quad = RL\ or\ SM\ at\ earlier\ harvest \\
&\quad + (RL\ or\ SM\ at\ later\ harvest - RL\ or\ SM\ at\ earlier\ harvest) \\
&\quad \div (actual\ date\ of\ later\ harvest\ - actual\ date\ of\ earlier\ harvest) \\
&\quad \times (average\ day\ of\ harvest - actual\ date\ of\ earlier\ harvest)
\end{aligned}
$$

For shoot variables, data from Harvests 1 to 4 was used. As previously noted, visibly degraded roots were removed during Harvest 2 processing. This meant that data from Harvest 2 was not fully comparable with data from other harvests. Due to non-comparability of root data from Harvest 2, values for root length were calculated based on data from Harvests 1, 3, and 4 only. Specifically, root variable values for days 183 and 230 were calculated using data from Harvests 1 and 3. Because RL data from Harvest 2 was not used, this likely generated greater error variation in root length values for days 183 and 230. Also, in 4 of 5 harvests, *E. palustris* at Superior north, the coldest site, demonstrated the highest RL, which could be attributed to slow decomposition of roots that may not have been eliminated from local substrate used to fill root ingrowth cores. As this site was an outlier in some calculations, the data were removed from statistical analyses, as indicated in the appropriate tables in the results.

Statistical tests were conducted to determine whether there were relationships between root length, soil temperature sum (GDD; January 1 to average dates of harvest), and root growth strategy, as well as between shoot dry mass with the same variables. Normality of the data was determined using the Shapiro-Wilk normality test. In some cases data was log transformed, to achieve normal distribution. Statistical tests used were Linear Mixed Effects Models. Dependent variables in the models included root length and shoot dry mass; independent variables included GDD at average harvest dates and strategy (multi- vs single-season root growth); a random independent factor was species nested within strategy. Specifically, the following dependent variables were included: early season root length (RL) and shoot dry mass (SM; day 183); RL and SM at midseason (day 230); relative late season RL and SM change (RL or SM at day 273 – RL or SM at day 230 ÷ RL or SM at day 230) to describe the species' senescence behaviour

independent of species size; maximum RL and SM (higher of values at day 230 or 273) to describe midseason or end of season growth of the species; and ratio of spring to maximum RL and SM (RL or SM at day 183 as a proportion of maximum) to describe early season growth as a proportion of maximum growth independently of species size.

Additionally, log transformed RL and SM at harvest dates 183 and 230 (spring and midseason) were analysed using backward stepwise general linear models starting with all combinations of the factorial variables species and harvest date, and the continuous variable transformed GDD $((GDD - 140)^{0.65})$ as independent variables, as well as interactions between the variables.

Root samples demonstrated inconsistent red staining after exposure to TTC solution. These results did not seem reliable, thus only the total measured root length was used in the analyses. For variables directly related to absolute root length and shoot dry mass, data from *S. emersum* on Manitoulin was left out, as this species is smaller in size than *S. americanum* at Superior (Flora of North America Editorial Committee 1993), and is indicated in appropriate tables and figures in the results. In two cases an outlier was left out (*E. palustris* at Superior north), as indicated in appropriate statistical output tables.

Calculations were performed in Microsoft Excel, Version 14 and statistical analyses were completed in R, Version 3.0.1 (R Core Team 2013).

Chapter 3: Results

3.1 Air and soil temperature

Average soil Growing Degree Days (GDD; 5 °C as baseline temperature) was highest at Manitoulin and lowest at Superior north (Table 3.1), with Manitoulin 409 degree days or 33% higher than Superior north, and differences between the three zones being significant ($p = 0.03$) in ANOVA models (Table 3.2, Appendix 5). As expected, the lowest annual soil GDD was observed at a Superior north site (*E. palustris*), and was 1153 degree days or 59% lower than the highest annual GDD, which was observed at the *E. palustris* site at Manitoulin (Appendix 6).

Differences in air temperature between the study zones from January to December 2014 were also significant ($p < 0.01$, Table 3.2), with the average GDD 59 degree days or 4% higher at Superior north than at Superior south (Table 3.1, Appendix 5), and average GDD 289 degree days or 18% higher at Manitoulin than at Superior north. However, data from 1 of the 2 aerial temperature sites at Manitoulin could not be tested due to missing data (10-day period in May 2014 at Misery Bay; Appendix 7).

A repeated measures ANOVA for spring, summer, and fall resulted in significant soil ($p < 0.04$) and air ($p = < 0.001$) temperature differences among the zones. The interaction between season and zone was not significant (Table 3.2). Differences in soil GDD between sites of species with multi-season roots (sMSR) and those of species with single-season roots (sSSR) were not significant, neither for the whole year nor when tested for seasonal differences (Table 3.2).

However, such differences were not expected due to a smaller number of replicates than required to determine whether there were statistically significant differences between sites.

Across all sites, aerial minimum temperatures during winter 2013 – 2014 were below -30 °C (Table 3.3). Minimum soil temperatures were above 0 °C at all sites except *T. cespitosum* at Superior north (-5 °C) and *S. latifolia* at Manitoulin (-2.5 ºC). Soil GDD at harvest dates is shown in Appendix 8.

Monthly soil GDD is shown by species in Figure 3.1. In general, soil temperature was higher and the growing season longer at Manitoulin compared to Superior, and at Superior south compared to Superior north. Notable observations were the very low temperatures at the *E. palustris* site at Superior north and the higher temperature at the *T. cespitosum* site at Superior south compared to Manitoulin. Monthly aerial GDD is shown in Figure 3.2. Of note are the lower temperatures during the summer months at Superior south sites compared to Superior north sites.

Figure 3.3 displays cumulative soil GDD at all sites, by harvest, for illustrative purposes only, as data was statistically analyzed based on average dates between harvests, and not on actual harvest dates. At each harvest, Superior north sites generally had the lowest temperature and Manitoulin sites the highest temperature, but there was some overlap between the study regions, which could be attributed to variation in harvest dates.

Table 3.1. Air and soil Growing Degree Days above 5 °C for the three zones in the study for the year 2014 and seasons in 2014.

Zone	Year 2014	Winter 1 (Jan-Mar)	Spring (Apr-Jun)	Summer (Jul-Aug)	Fall (Sep-Oct)	Winter 2 (Nov-Dec)
Air temperatures						
Superior north	1316 ± 30	0.01 ± 0.01	456 ± 14	612 ± 11	245 ± 6	2 ± 0.3
Superior south	1257 ± 20	0.6 ± 1	435 ± 36	568 ± 8	251 ± 15	4 ± 0.7
Manitoulin	[a]	0 ± 0	[a]	719 ± 23	366 ± 3	7 ± 0.5
Soil temperatures						
Superior north	1251 ± 113	0.2 ± 0.2	274 ± 28	633 ± 64	329 ± 40	14 ± 8
Superior south	1547 ± 82	0 ± 0	352 ± 32	754 ± 35	430 ± 25	12 ± 4
Manitoulin	1660 ± 114	0 ± 0	364 ± 22	741 ± 58	512 ± 36	43 ± 9

Mean values of sites within zones ±1SE.

[a] Data not used, as values missing for May 13-23, 2014 from Misery Bay aerial temperature dataloggers.

Table 3.2. ANOVA with Soil and Air GDD in 2014 and during growing season (Apr – Oct) as dependent variables; zone (Superior north, Superior south, Manitoulin), strategy (multi- or single-season roots), and season (spring Apr – Jun; summer Jul – Aug; fall Sep – Oct) as independent factors; and zone x season, and zone x strategy as independent interaction effects.

Statistical test	Dependent variable	Independent variable	df	Mean Square	F	P
ANOVA	Soil GDD 2014	Zone	2	234104	4.6	0.033*
ANOVA	Soil GDD 2014	Zone	2	253706	5.0	0.029*
		Strategy	1	7128	0.1	0.716
ANOVA	Air GDD 2014	Zone	2	60676	30.9	0.004**
Repeated Measures ANOVA	Soil GDD (Apr-Oct)	Zone	2	71020	4.5	0.035*
		Season	2	561834	147.0	< 0.001**
		Zone×Season	4	4750	1.2	0.320
Repeated Measures ANOVA	Soil GDD (Apr-Oct)	Zone	2	71020	4.9	0.038*
		Strategy	1	16534	1.0	0.329
		Season	2	561834	140.2	< 0.001**
		Zone×Season	4	4750	1.2	0.345
		Strategy×Season	2	1802	0.45	0.643
Repeated measures ANOVA	Air GDD (Apr-Oct)	Zone	2	26108	28.8	< 0.001**
		Season	2	238245	263.0	< 0.001**
		Zone×Season	4	673	0.74	0.579

*significant **highly significant

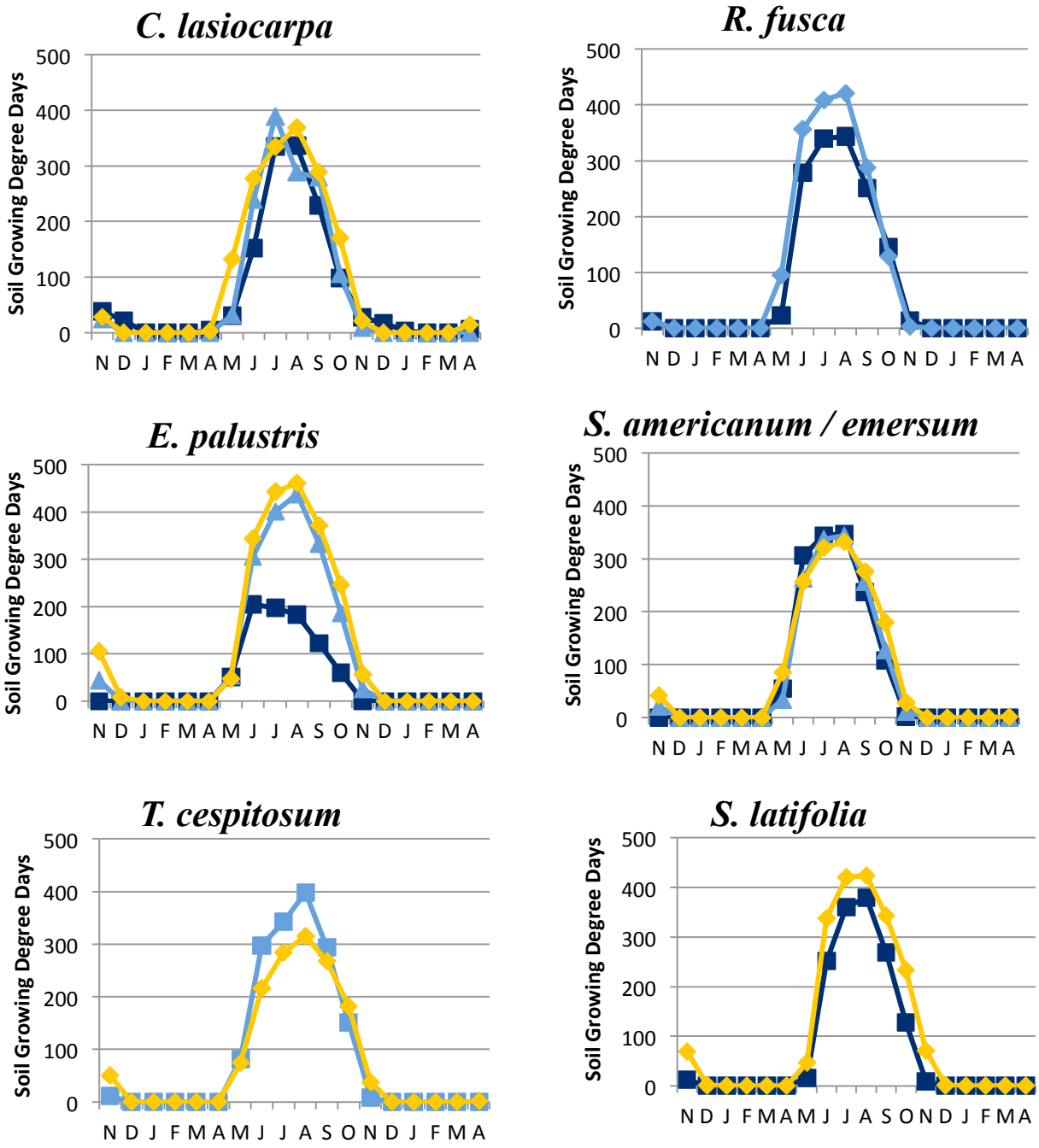

Figure 3.1. Monthly soil Growing Degree Days above 5 ºC, by species.

Study period November 2013 to April 2015. Sites at Superior north, Superior south, and Manitoulin shaded dark blue, light blue, and yellow, respectively. n=12 (*C. lasiocarpa*), 9 (*E. palustris*), 8 (*T. cespitosum*), 8 (*R. fusca*), 12 (*S. americanum/emersum*), 4 (*S. latifolia*). November 2013 data begins November 3 (latest date of core installation across all sites).

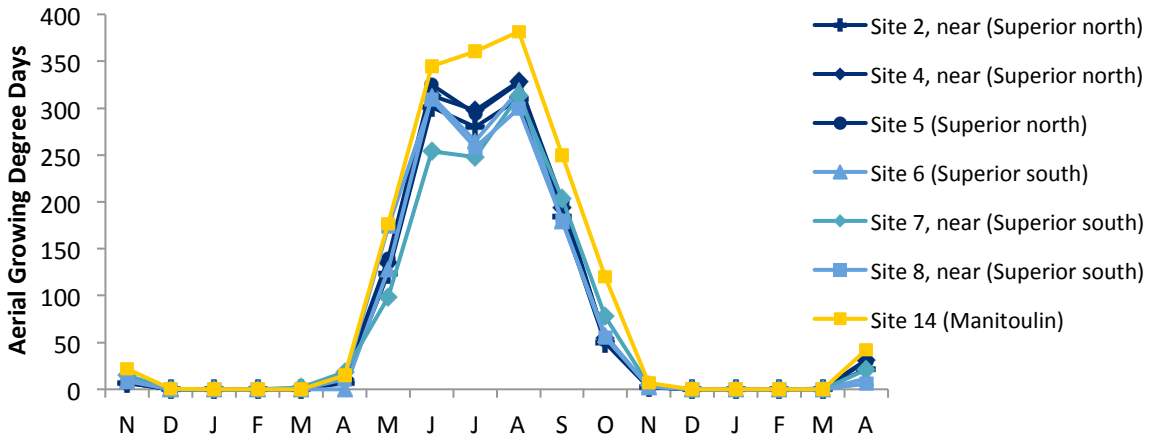

Figure 3.2. Monthly aerial Growing Degree Days above 5 ºC.

Study period November 2013 to April 2015. Aerial temperature data from Misery Bay excluded as values from May 13-23, 2014 were missing due to datalogger malfunction. Temperature dataloggers were installed at site or near sites, as indicated in the legend. Sites at Superior north, Superior south, and Manitoulin represented by lines shaded dark blue, light blue, and yellow, respectively. n=11, 2 at Superior and Manitoulin, respectively. See Figures 2.3 and 2.4 for a map of study sites (Methods section 2.2).

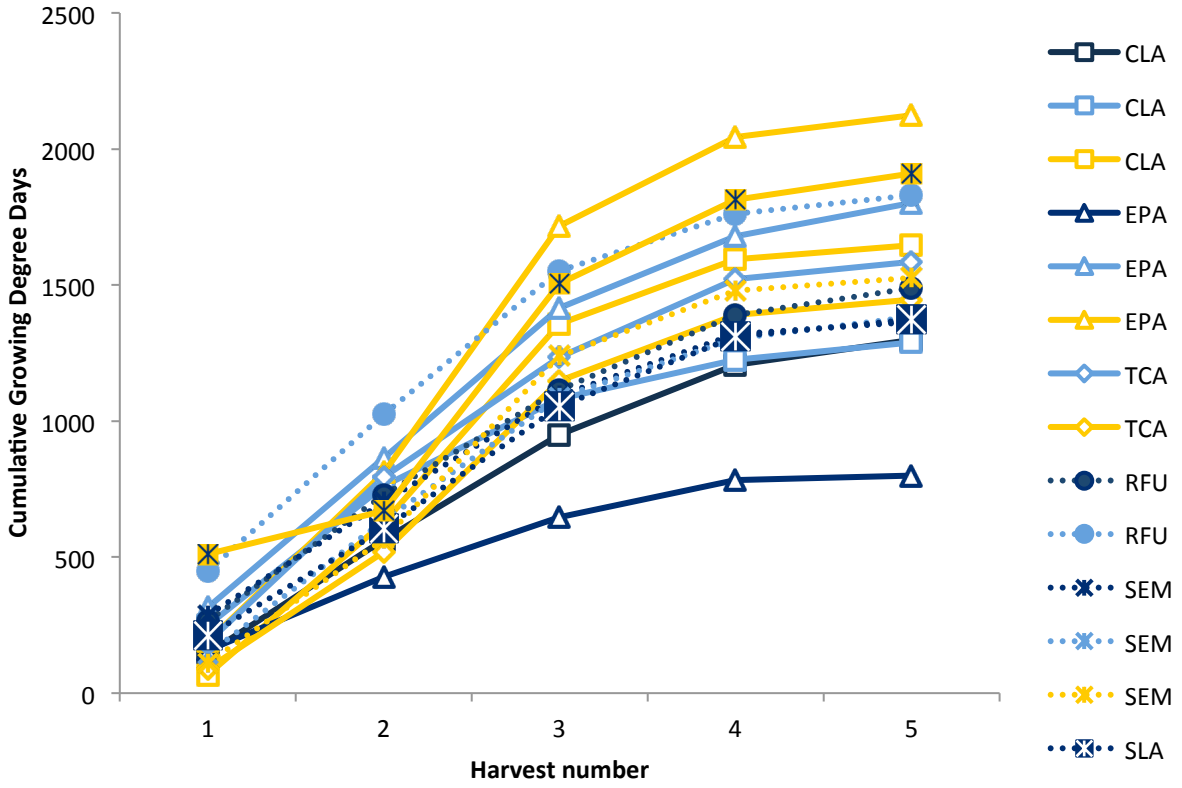

Figure 3.3. Cumulative soil Growing Degree Days above 5 ºC at all sites at each harvest.

Sites at Superior north, Superior south, and Manitoulin shaded dark blue, light blue, and yellow, respectively. Species abbreviations are as follows. CLA: *C. lasiocarpa*; EPA: *E. palustris*; TCA: *T. cespitosum*; RFU: *R. fusca*; SEM: *S. emersum, S. americanum* (treated as one species); SLA: *S. latifolia*. n=29, 24 for sites at species with multi- and single-season roots; 27, 18 for sites at Superior and Manitoulin, respectively. Harvest dates varied among sites (see Appendices 10, 12).

Table 3.3. Minimum soil and aerial temperatures (°C) measured at study sites from November 2013 to March 2014.

Location	Latitude (° North)	Species	**Soil temperature**			
			Replicate 1		Replicate 2	
			20 cm depth	10 cm depth	20 cm depth	10 cm depth
Lake Superior Provincial Park						
1 (Rabbit Blanket Lake)	47.75	*S. americanum*	0.5			0.5
2 (Rabbit Blanket Lake)	47.75	*S. latifolia*	1.0	1.0		
3 (Rabbit Blanket Lake)	47.75	*E. palustris*	2.5	2		1
4 (Red Rock Lake)	47.72	*C. lasiocarpa*		0.5		
5 (Mijinemungshing Lake)	47.70	*R. fusca*	1	0.5	1	0
6 (Trapper's Trail)	47.54	*S. americanum*	1.5	1	1.5	1
7 (Coldwater)	47.47	*T. cespitosum*	-0.5	-2	-5	-5
8 (MacGregor Lake)	47.28	*C. lasiocarpa*	1.5	1.5		
9 (Crescent Lake)	47.28	*E. palustris*	0.5	1	0.5	0.5
10 (Crescent Lake)	47.28	*R. fusca*	0.5	0	0	0
Manitoulin Island						
11 (Misery Bay)	45.79	*C. lasiocarpa*	1.5	1.5	1	0.5
12 (Misery Bay)	45.80	*T. cespitosum*	1	1	1	1
13 (Michael's Bay)	45.61	*S. emersum*	1	1		
14 (Snow Lake)	45.68	*E. palustris*	0.5	0	1	0.5
15 (Snow Lake)	45.68	*S. latifolia*	-2	-1	-1	-2.5

			Aerial temperature			
			Replicate 1	Replicate 2		
Lake Superior Provincial Park						
2 (Rabbit Blanket Lake)	47.75		-39.5	-39.5		
4 (Red Rock Lake)	47.72		-36.5	-36.0		
5 (Mijinemungshing Lake)	47.70		< -40.0	< -40.0		
6 (Trapper's Trail)	47.54		-34.5	-34.5		
7 (Agawa Bay)	47.33		-32.5	-33.0		
8 (Crescent Lake)	47.28		-36.0	-35.5		
Manitoulin Island						
12 (Misery Bay)	45.80		-36.5	-36.5		
14 (Snow Lake)	45.68		-34	-34		

Lower measurement limit for dataloggers was -40 °C. Blank cells in soil temperature table indicate missing data.

3.2 Root length

Root length (RL; cm) results at each harvest are presented, using median RL values of all species. At Harvest 1, average RL of species with single-season roots (sSSR) was 73% lower than average RL of species with multi-season roots (sMSR). When considering sites at Superior only, RL of sSSR was 81% lower than RL of sMSR. When considering sites at Manitoulin only, RL of sSSR was 22% lower than RL of sMSR. As the method of processing roots from Harvest 2 was different than in the other harvests, the results are not comparable across harvests. At Harvest 2, RL of sSSR was 45% lower than RL of sMSR. At Superior, RL of sSSR was 26% lower than RL of sMSR. At Manitoulin, RL of sSSR was 92% lower than RL of sMSR.

At Harvest 3, RL of sSSR was 45% lower than RL of sMSR. At Manitoulin, RL of sMSR was nearly three times that of sSSR, while at Superior, RL of sMSR was 18% lower than that of sSSR. At Harvest 4, RL of sSSR at Superior was 33% lower than that of sMSR, while at Manitoulin, sSSR had RL 41% lower than sMSR. RL of sMSR did not fluctuate greatly after Harvest 3. For both regions, RL at Harvest 4 was similar to that at Harvest 5, indicating little growth or decomposition during the winter of 2014-2015 (Figure 3.4).

As the harvest dates differed for each site, statistical analyses were conducted only for calculated RL at average dates of harvest (average dates between successive harvests across all sites). At day 183 (July 2), RL was not affected by root growth strategy (multi- vs. single-season) nor by soil Growing Degree Days (GDD) since January 1 (Table 3.4, Figure 3.5). However, at a given soil GDD, RL at day 183 was generally lower for sSSR.

At day 230 (August 18), there was a positive relationship between RL and soil GDD, with *E. palustris* at Superior north as an outlier (Figure 3.6). The relationship between RL and GDD on day 230 was significant ($p < 0.02$) only when data from this site were excluded (Table 3.4). Despite being the site with the coldest soil temperature, this site demonstrated the highest root length across all harvests, with the exception of *C. lasiocarpa* at Manitoulin at Harvest 5 (Fig. 3.4). This could be explained by an abundance of undecomposed dead roots.

There were no significant temperature or strategy effects on the relative change of root length at the end of the season, which is to say RL change from day 230 to day 273 (August 18 to September 30) in relation to RL at day 230 (Figure 3.7). Species with MSR demonstrated higher median relative RL change in fall than sSSR, but the difference was statistically non-significant.

Maximum RL (higher of RL values at day 230 or 273) increased with soil GDD during the growing season (GDD at day 273), but also here, the relationship was significant only when RL data for *E. palustris* at Superior north (the coldest site) was excluded ($p = 0.02$; Figure 3.8). There was no relationship between maximum RL and strategy.

The ratio of RL at day 183 to maximum RL was not significantly affected by temperature or by strategy (Fig. 3.9). Species with MSR demonstrated higher median spring to maximum RL than sSSR, but the difference was statistically non-significant.

When RL at days 183 and 230 were analysed with a stepwise general linear model, all three factors—harvest date, species, and GDD—had significant effects ($p < 0.01$). Additionally, the interaction factor harvest date × GDD was weakly significant ($p = 0.05$) and the interaction factor harvest date × species × GDD was significant ($p < 0.01$). Figures 3.6 and 3.8 illustrate the

generally positive relationship between RL and GDD, but the significant interactions indicate that there was some variation in this relationship depending on species and harvest date (Table 3.5).

Statistically analyzed root length and temperature data from average harvest dates are in Appendix 9. Root length data from actual harvest dates are contained in Appendix 10.

It should be repeated that roots that appeared to be dead due to degraded, limp, or frayed appearance were discarded during root length measurement for Harvest 2; results from Harvest 2 should therefore be treated with caution. The TTC solution that was intended to determine living root length stained roots in an unreliable manner. Variation in staining was sometimes large among replicates of the same species from the same site. Staining was considered inconclusive and results of staining were not tested for statistical significance. The data are included in Appendix 11.

It should also be repeated that during the soil sieving stage of the initial installation of root ingrowth cores, it is likely that some dead and undecomposed roots were not eliminated. This may have influenced root length measurements and was likely most pronounced in the first harvest in the spring of 2014. This would also have influenced root length data in the fifth and final harvest in the spring of 2015, as roots that senesced in fall 2014 were likely still present in spring 2015. Results from Harvest 5 were not statistically analyzed.

Figure 3.4. Median root length (cm) at sites of species with multi-season roots and single-season roots by date of harvest.

Multi-season roots and single-season roots represented by filled and open symbols, respectively. For values from the final harvest, 365 days were added to dates, to indicate that they occurred the following year. Sites in northern and southern zones of Superior and at Manitoulin represented by symbols ◊, □, and ○, respectively. Species abbreviations are as follows. CLA: *C. lasiocarpa*; EPA: *E. palustris*; TCA: *T. cespitosum*; RFU: *R. fusca*; SEM: *S. emersum, S. americanum* (treated as one species); SLA: *S. latifolia*. n=55 (CLA), 60 (EPA), 37 (TCA), 41 (RFU), 55 (SEM), 35 (SLA). Y axis log transformed.

Table 3.4. Nested linear mixed effects models with Root Length (RL; cm) at days 183 and 230, RL change from days 230 to 273, RL maximum, and ratio of RL at day 183 to RL maximum as dependent variables; strategy and soil GDD above 5 ºC at days 183, 230, and 273 as independent factors; and species within strategy as nested random factor.

	df	Mean square	F	P
RL 183				
AIC: 36.6				
Strategy	1	4	0.6	0.475
GDD 183	1	7	0.003	0.958
RL 230[1]				
AIC: 26.4				
Strategy	1	4	0.0	0.975
GDD 230	1	6	10.5	0.017*
RL change 230-273				
AIC: 26.4				
Strategy	1	4	2.1	0.243
GDD 273	1	8	0.5	0.502
RL maximum[2]				
AIC: 24.6				
Strategy	1	4	0.5	0.579
GDD 273	1	6	8.7	0.022*
RL 183 : RL maximum				
AIC: 23.8				
Strategy	1	4	0.0	0.919
GDD 183	1	8	1.0	0.352

n=62, 54; 62, 55; 61, 53 for species with multi- and single-season roots at days 183, 230, and 273, respectively. *S. emersum* at Manitoulin removed from analysis except in tests of RL change 230-273 and RL 183 : RL maximum. [1,2] *E. palustris* at Superior north outliers removed.
*significant

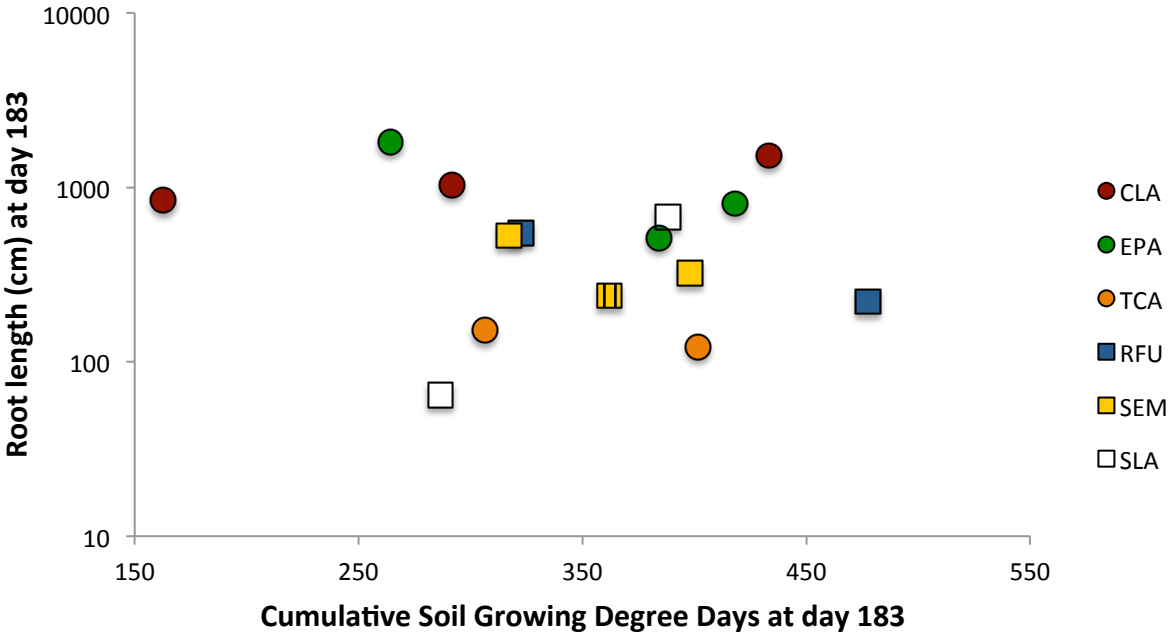

Figure 3.5. Early season root length (cm) across all sites on day 183 (July 2) against soil cumulative Growing Degree Days above 5 °C at day 183.

Calculation is based on median RL values at harvests 1 and 3. Species with multi-season roots and species with single-season roots represented by circle and square symbols, respectively. *S. emersum* (Manitoulin) was not included in the statistical analysis (striped pattern). Y axis log-transformed.

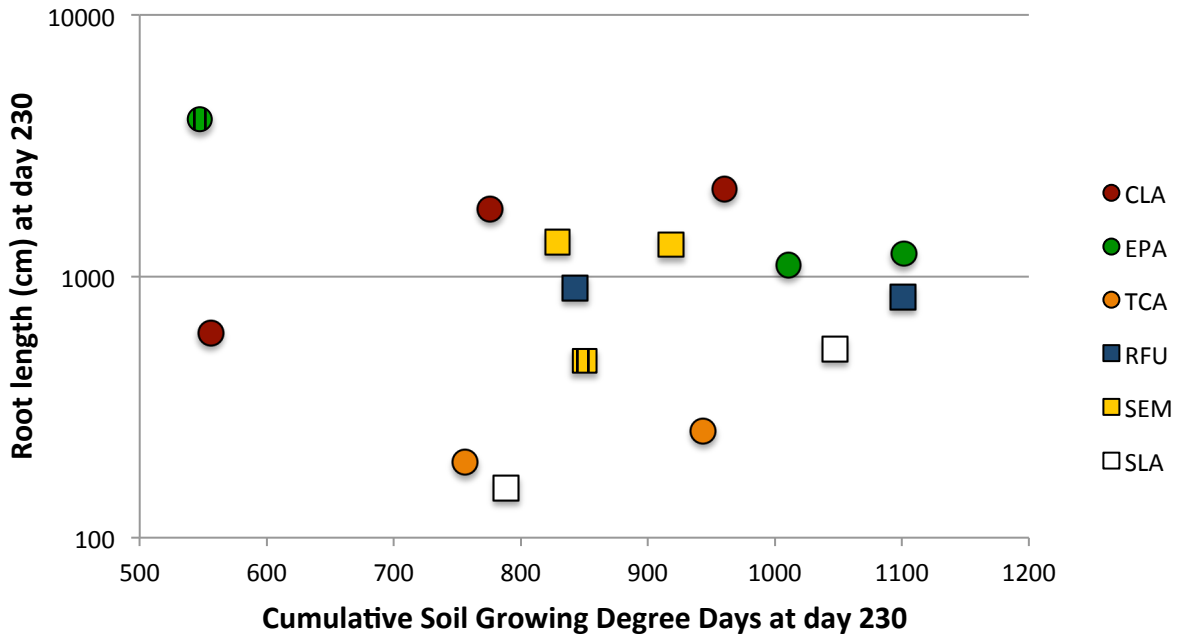

Figure 3.6. Midseason root length (cm) across all sites on day 230 (August 18), against cumulative soil Growing Degree Days above 5 °C at day 230.

Calculation is based on median values at harvests 1 and 3. Species with multi-season roots and species with single-season roots represented by circle and square symbols, respectively. *S. emersum* (Manitoulin) and *E. palustris* (Superior north) were not included in the statistical analysis (striped pattern). Y axis log-transformed.

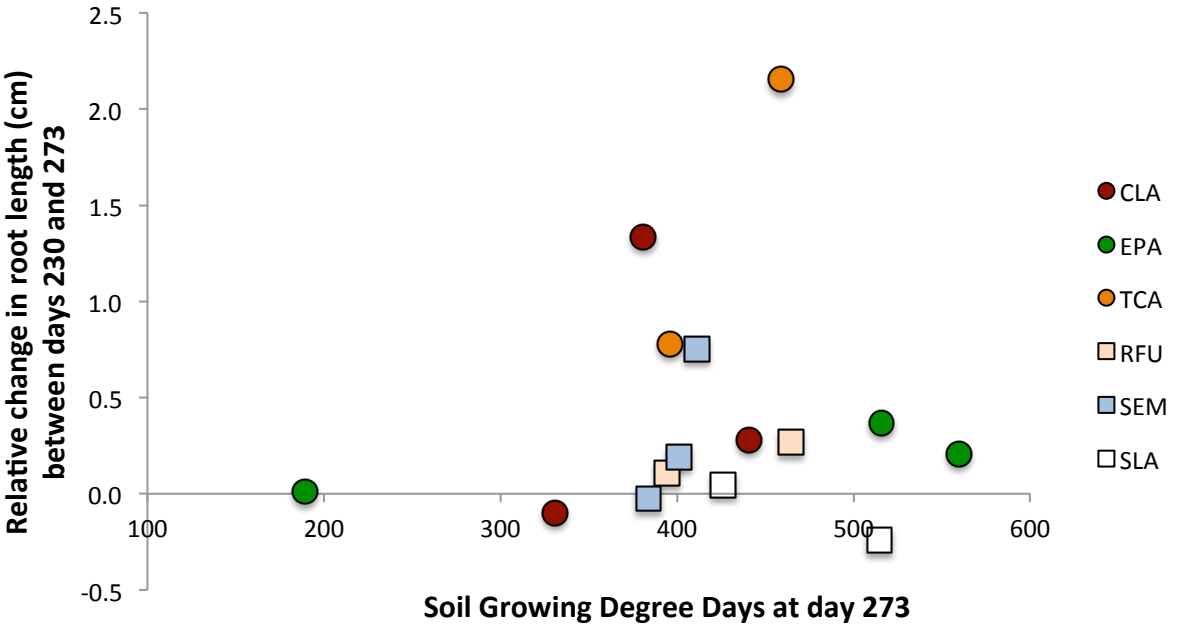

Figure 3.7. Late season root length (cm) change, expressed as the proportional change in root length across all sites between days 230 and 273 (August 18 and September 30) against soil Growing Degree Days above 5 °C from day 230 to 273.

Calculation is based on median values at harvests 1, 3 and 4. Species with multi-season roots and species with single-season roots represented by circle and square symbols, respectively.

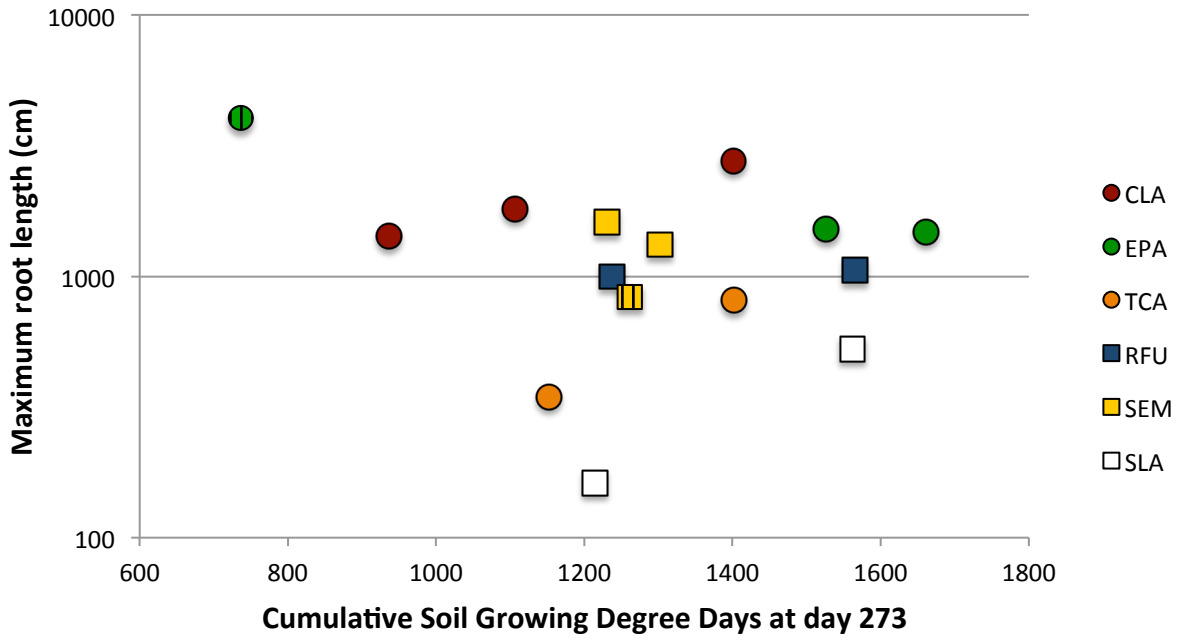

Figure 3.8. Maximum root length (cm), expressed as the higher root length value at day 230 (August 18) or 273 (September 30) across all sites against the cumulative soil Growing Degree Days above 5 °C at day 273 (September 30).

Calculation is based on median values at harvests 1, 3 and 4. Species with multi-season roots and species with single-season roots represented by circle and square symbols, respectively. *S. emersum* (Manitoulin) and *E. palustris* (Superior north) were not included in the statistical analysis (striped pattern). Y axis log-transformed.

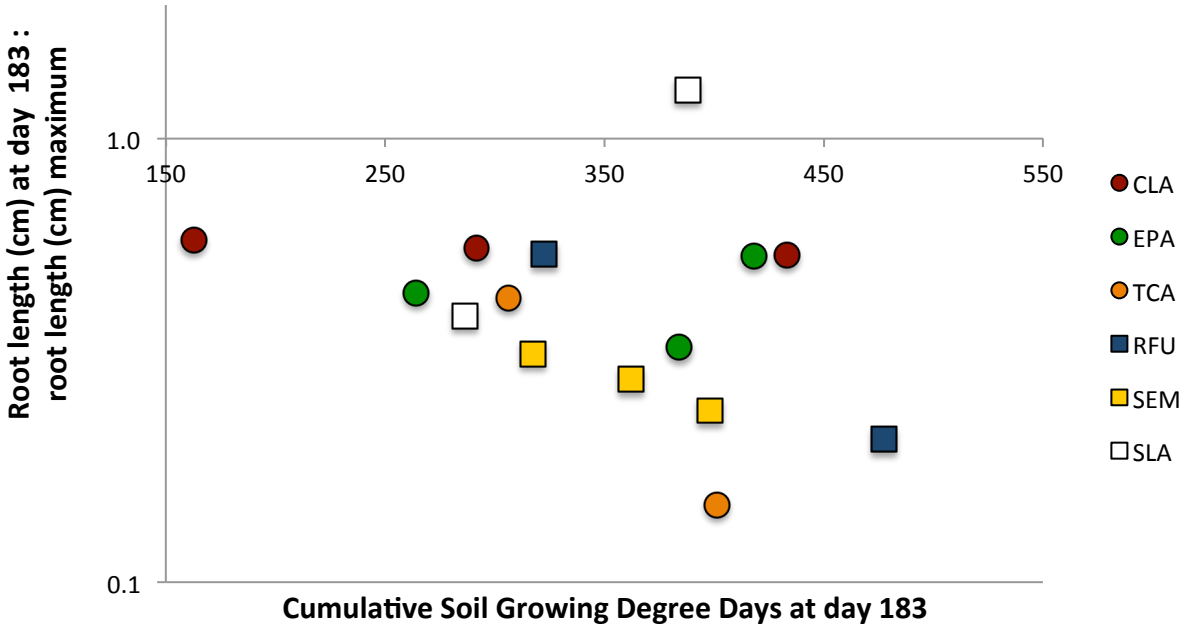

Figure 3.9. Relative early season root growth, expressed as the ratio of root length (cm) across all sites on day 183 (July 2) to maximum root length (day 230 [August 18] or 273 [September 30]) against the cumulative soil Growing Degree Days above 5 °C at day 183.

Calculation is based on median values at harvests 1, 3 and 4. Species with multi-season roots and species with single-season roots represented by circle and square symbols, respectively. Y axis log-transformed.

Table 3.5. Stepwise general linear model with log transformed Root Length (RL; cm) at days 183 and 230 as the dependent variable; harvest date, species, and transformed GDD ((GDD – 140)$^{0.65}$) as independent factors; and harvest date × GDD and harvest date × species × GDD as interaction factors.

	df	Mean Square	F	P
RL 183 or 230				
$r^2 = 0.94$				
Harvest date	1	0.4	40.5	< 0.001
Species	5	0.5	47.1	< 0.001
GDD	1	0.2	20.7	0.003
Harvest date × GDD	1	0.1	5.6	0.050
Harvest date × Species × GDD	10	0.1	7.9	0.006

3.3 Shoot dry mass

Shoot dry mass (SM; mg) results at each harvest are presented, using median SM values of all species. For sMSR, average SM at Harvest 1 was 32% lower than average SM of sSSR. Harvest 2 was the only harvest for which SM of sSSR was lower than that of sMSR, in this instance by 44%. For sMSR, SM at Harvest 3 was 42% lower than SM of sSSR. For sMSR, SM at Harvest 4 was 54% lower than SM of sSSR. Across all harvests, at Superior, SM of sMSR was 77% lower than that of sSSR; at Manitoulin, SM of sMSR was 65% lower than that of sSSR. In all harvests except Harvest 2, sSSR demonstrated higher SM. Species at Superior had higher SM than those at Manitoulin, and generally, species at Superior north had higher SM than those at Superior south (Figure 3.10).

As the harvest dates differed for each site, statistical analyses were conducted only for calculated SM at average dates of harvest (average dates between successive harvests across all sites). At day 183, shoot growth was negatively influenced by soil temperature ($p = 0.01$), as overall Shoot dry Mass (SM) was lower at sites with higher soil GDD (Table 3.6, Figure 3.11).

At day 230, there was no significant relationship between shoot dry mass and GDD (Figure 3.12). These results indicate that, by midseason, shoot growth of plants in warmer environments had caught up to that of plants in cooler environments. There were no relationships between SM at days 183 and 230 with root growth strategy.

Relative late season change in SM from day 230 to day 273 was not dependent on soil temperature, but the median value was significantly higher ($p = 0.03$) for sSSR than for sMSR

(increase in SM for sSSR, decrease in SM for sMSR; Figure 3.13). This reflects the differences between strategy types in the partitioning of resources prior to the onset of winter.

The generally negative relationship between maximum SM (higher of SM values at day 230 or 273) and GDD was not significant. There was also no relationship between maximum SM and strategy (Figure 3.14).

The ratio of SM at day 183 to maximum SM did not respond to soil temperature, but strategy had a nearly significant effect ($p = 0.053$), with sSSR demonstrating lower median values than sMSR (Figure 3.15). The implication is that sSSR experience delayed shoot growth.

When SM at days 183 and 230 was analysed with a stepwise general linear model, effects of harvest date and species were highly significant ($p < 0.01$) and the effect of GDD was significant ($p = 0.01$). Additionally, the interaction factor species × GDD was weakly significant ($p = 0.05$). Figure 3.11 illustrates the negative relationship between SM and GDD, but the significant interaction indicates that there was some variation in this relationship, depending on species (Table 3.7).

Statistically analyzed shoot dry mass and temperature data from average harvest dates are in Appendix 9. Shoot dry mass data from actual harvest dates are contained in Appendix 12.

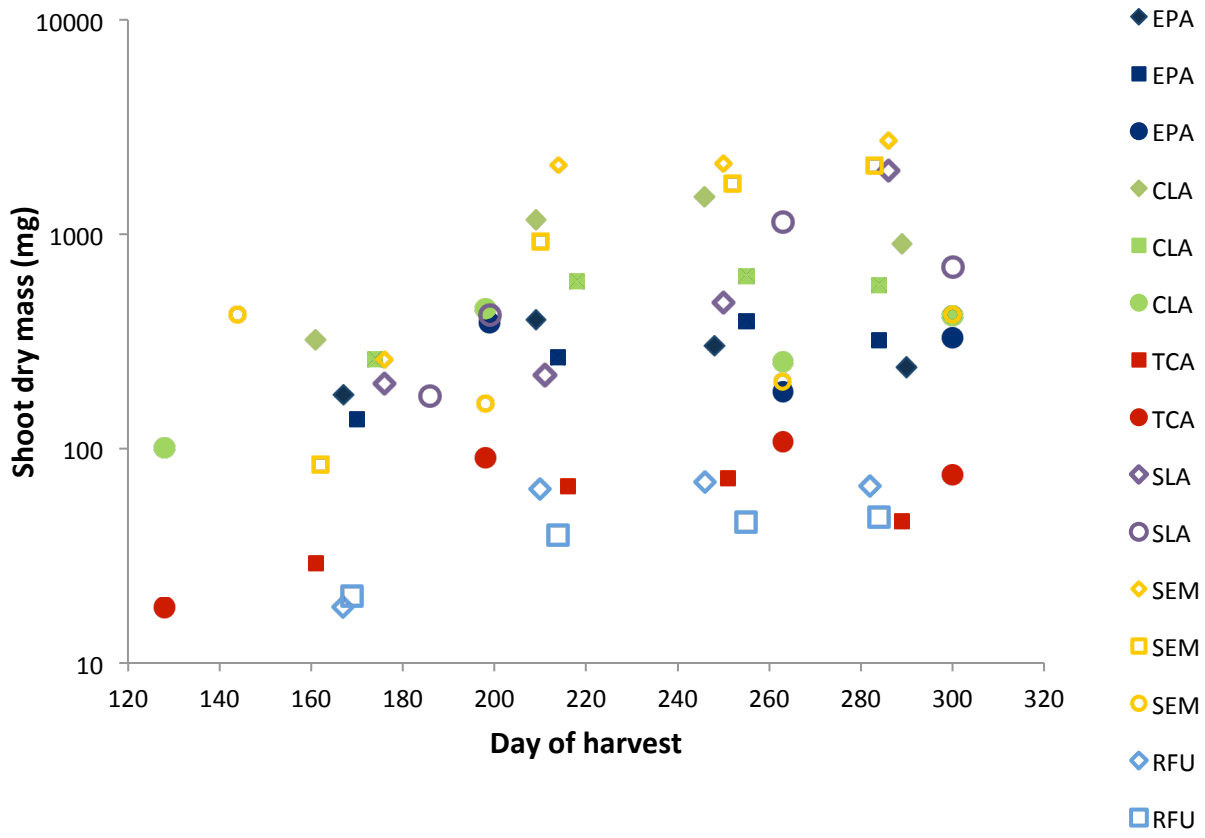

Figure 3.10. Median shoot dry mass (mg) at sites of species with multi-season and single-season roots by day of harvest.

Multi-season and single-season roots represented by filled and open symbols, respectively. Sites in northern and southern zones of Superior and at Manitoulin represented by symbols ◊, □, and ○, respectively. Species abbreviations are as follows. CLA: *C. lasiocarpa*; EPA: *E. palustris*; TCA: *T. cespitosum*; RFU: *R. fusca*; SEM: *S. emersum*, *S. americanum*, treated as one species; SLA: *S. latifolia*. n=114 (CLA), 105 (EPA), 82 (TCA), 74 (RFU), 111 (SEM), 34 (SLA). There was no shoot growth for *E. palustris* at Manitoulin at Harvest 1. Y axis log-transformed.

Table 3.6. Nested linear mixed effects model with Shoot dry Mass (SM; mg) at days 183 and 230, SM change from days 230 to 273, SM maximum, and ratio of SM at day 183 to SM maximum as dependent variables; strategy and soil GDD above 5 ºC at days 183, 230, and 273 as independent factors; and species within strategy as nested random factor.

	df	Mean square	F	P
SM 183				
AIC: 22.0				
Strategy	1	4	0.0	0.870
GDD 183	1	6	13.8	0.011*
SM 230				
AIC: 33.8				
Strategy	1	4	0.1	0.780
GDD 230	1	7	1.7	0.236
SM change 230-273				
AIC: 26.6				
Strategy	1	4	11.8	0.025*
GDD 273	1	8	0.2	0.636
SM maximum				
AIC: 32.2				
Strategy	1	4	0.4	0.598
GDD 273	1	7	3.4	0.106
SM 183 : SM maximum				
AIC: 21.6				
Strategy	1	4	9.0	0.053§
GDD 183	1	7	1.7	0.235

n=136, 101; 160, 120; 160, 118 for species with multi- and single-season roots at days 183, 230, and 273, respectively. *S. emersum* at Manitoulin removed from analysis except in tests of SM change 230-273 and SM 183 : RL maximum. *significant, §noteworthy

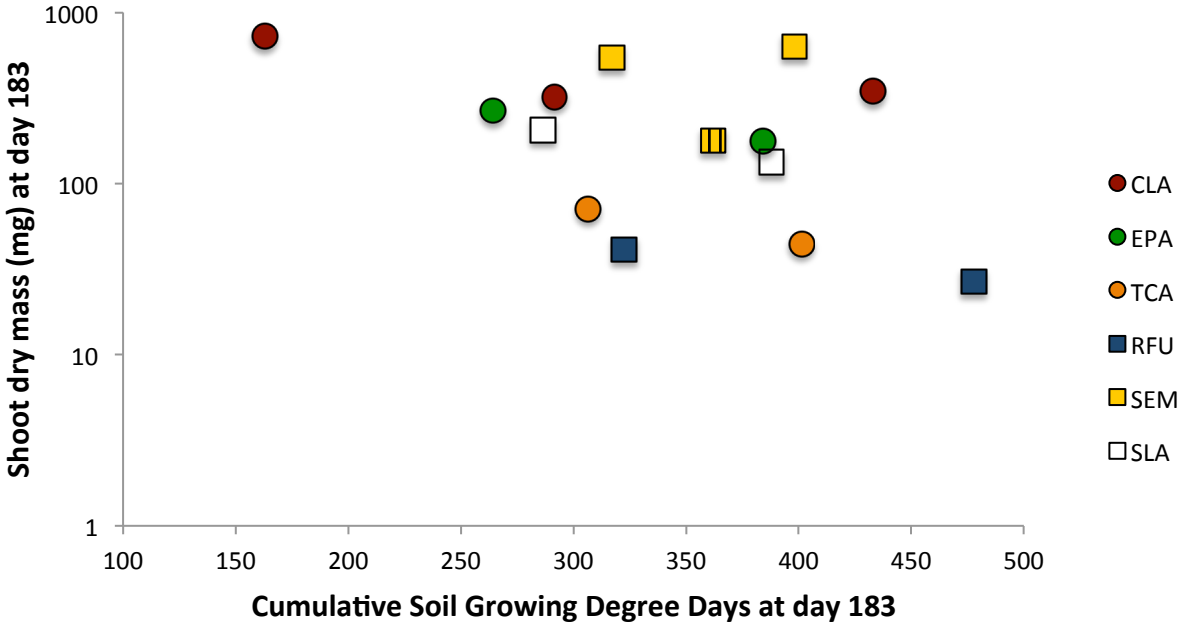

Figure 3.11. Early season shoot dry mass (mg) across all sites at day 183 (July 2) against the cumulative soil Growing Degree Days above 5 °C at day 183.

Calculation is based on median values at harvests 1 and 2. Species with multi-season and single-season roots represented by circle and square symbols, respectively. Data for *E. palustris* (Manitoulin) is missing (no shoot growth at Harvest 1). *S. emersum* (Manitoulin) was not included in the statistical analysis (striped pattern). Y axis log-transformed.

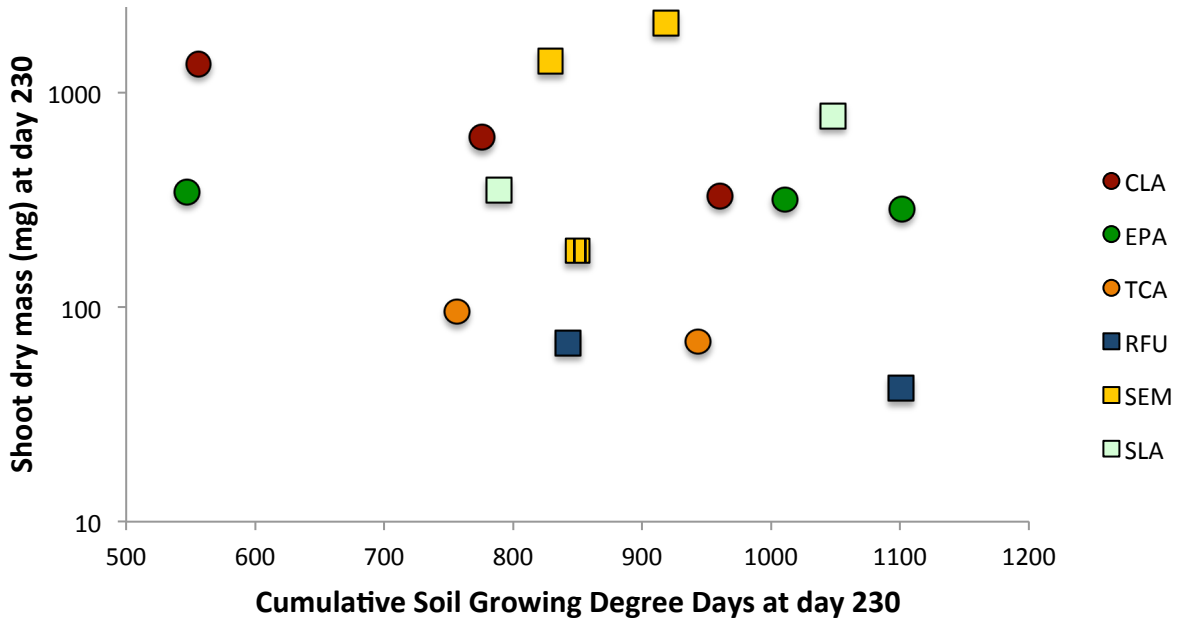

Figure 3.12. Midseason shoot dry mass (mg) across all sites at day 230 (August 18) against the cumulative soil Growing Degree Days above 5 °C at day 230.

Calculation is based on median values at harvests 2 and 3. Species with multi-season and single-season roots represented by circle and square symbols, respectively. *S. emersum* (Manitoulin) was not included in the statistical analysis (striped pattern). Y axis log-transformed.

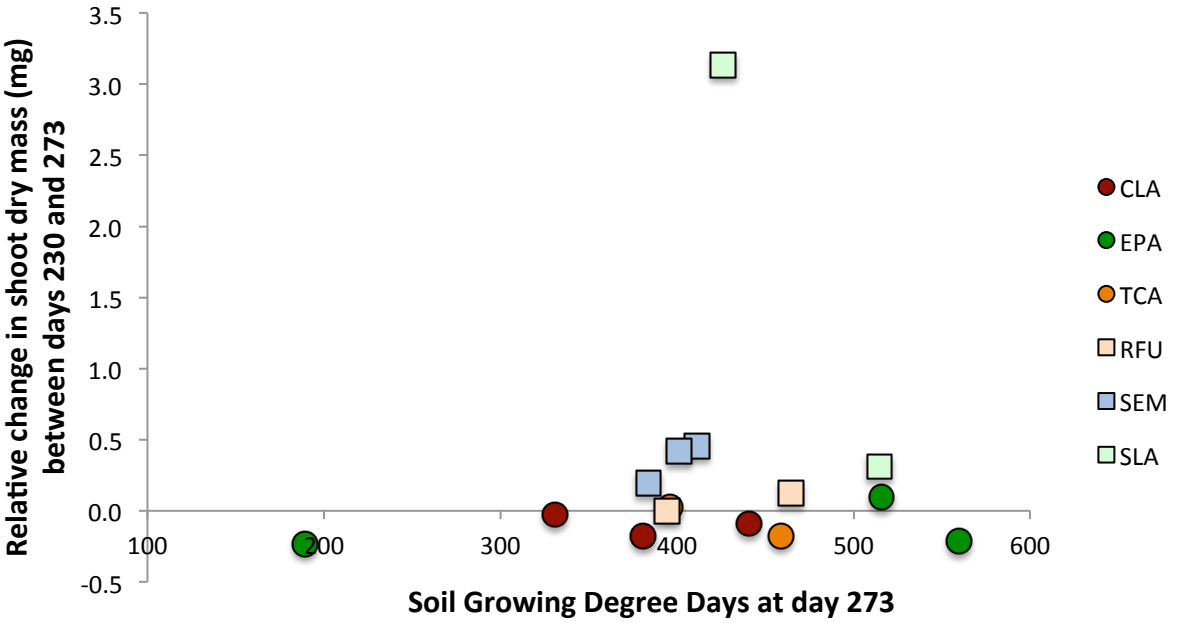

Figure 3.13. Late season shoot dry mass (mg) change, expressed as the proportional change in shoot dry mass across all sites between days 230 and 273 (August 18 and September 30) against soil Growing Degree Days above 5 °C from day 230 to 273.

Calculation is based on median values at harvests 2, 3 and 4. Species with multi-season and single-season roots represented by circle and square symbols, respectively.

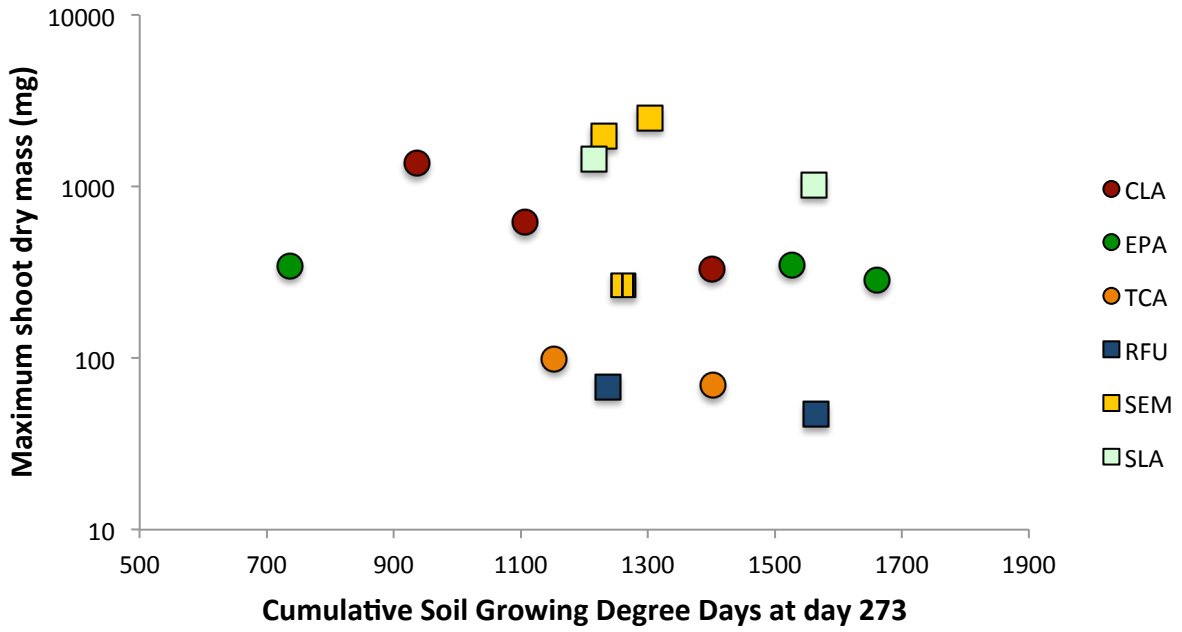

Figure 3.14. Maximum shoot dry mass (mg), expressed as the higher shoot dry mass value at day 230 (August 18) or 273 (September 30) across all sites against cumulative soil Growing Degree Days above 5 °C at day 273.

Calculation is based on median values at harvests 2, 3 and 4. Species with multi-season and single-season roots represented by circle and square symbols, respectively. *S. emersum* (Manitoulin) was not included in the statistical analysis (striped pattern). Y axis log-transformed.

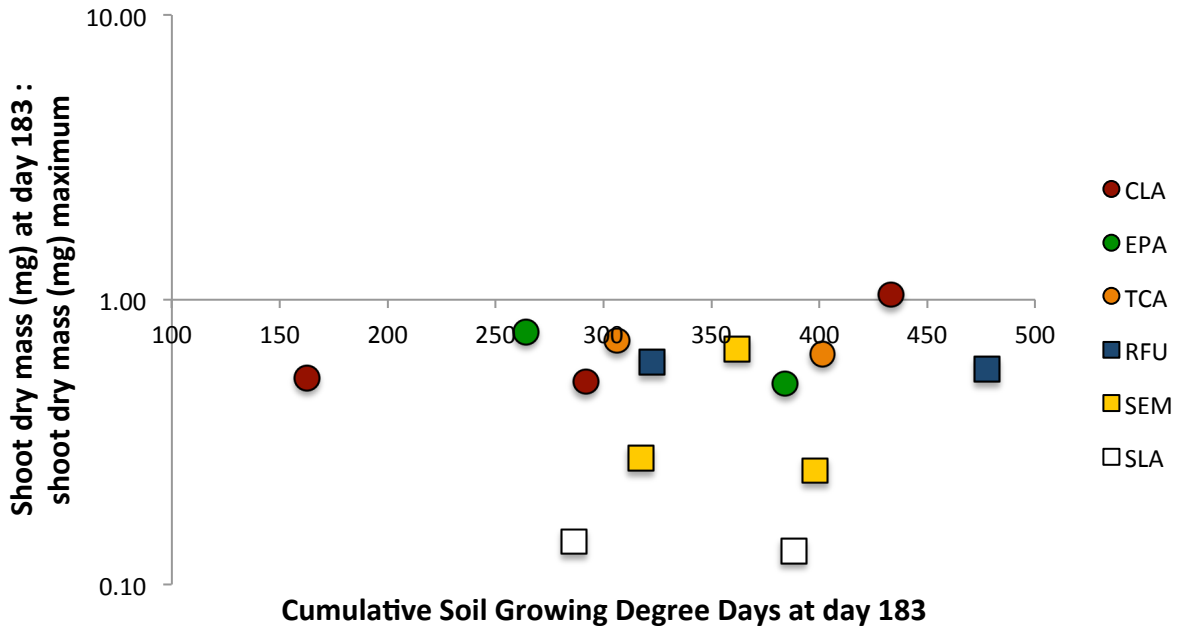

Figure 3.15. Relative early season shoot growth, expressed as the ratio of shoot dry mass (mg) across all sites on day 183 (July 2) to the maximum shoot dry mass (day 230 [August 18] or 273 [September 30]) against cumulative soil Growing Degree Days above 5 °C at day 183.

Calculation is based on median values at harvests 1, 2, 3 and 4. Species with multi-season and single-season roots represented by circle and square symbols, respectively. There was no shoot growth for *E. palustris* at Manitoulin at Harvest 1. Y axis log-transformed.

Table 3.7. Stepwise general linear model with log transformed Shoot dry Mass (SM; mg) at days 183 and 230 as the dependent variable; harvest date, species, and transformed GDD ((GDD – 140)$^{0.65}$) as independent factors; and species × GDD as an interaction factor.

	df	Mean Square	F	P
SM 183 or 230				
$r^2 = 0.94$				
Harvest date	1	0.5	31.4	< 0.001
Species	5	1.2	73.7	< 0.001
GDD	1	0.1	8.8	0.010
Species × GDD	5	0.0	2.9	0.0546

Chapter 4: Discussion

4.1 Temperature gradient

In this study, soil and aerial temperature differed significantly among the study zones, with Manitoulin demonstrating warmer temperatures than Superior. Generally, in North America, the further north a site is located, the shorter the growing season, and the less competitive are deciduous plants in comparison to evergreen. While the growing season is known to be longer at Manitoulin Island than at Lake Superior Provincial Park (Agriculture and Agri-food Canada 2014), differences in soil and aerial temperature between sites were important in this study, as these differences create functionally different growing season lengths. It has been acknowledged that root phenology differs among sites and species (Abramoff and Finzi 2015, Radville et al. 2016, Sloan et al. 2016). In their review of studies evaluating the impacts of climate change on root phenology, Radville et al. (2016) indicated that the timing of root growth may be related to soil temperature and carbon availability, and that maximal root growth may be related to plant resource allocation.

There were significant temperature differences between study zones. Manitoulin had warmer soil temperature than Superior, and sites at Superior south had warmer soil temperature than sites at Superior north. The four sites with the lowest soil GDD by September 30 were occupied by species with multi-season roots (sMSR), indicating that species with single-season roots (sSSR) are not well adapted to sites with cold temperature. Especially at Superior north (i.e., the region with the coldest soil temperatures), sSSR occupied warmer sites than sMSR. However, several

sites with sMSR were among those with the warmest soil temperatures, resulting in a lack of significant differences between average soil temperature at sites of sMSR and sites of sSSR.

There were also significant differences in aerial temperature between the zones, with temperatures significantly warmer at Superior north than Superior south. This could be attributed to the closer proximity of sites in Superior south to Lake Superior, a large and cold water body (Bennett 1978), compared to sites at Superior north which were farther inland. Aerial temperature was also warmer at Manitoulin than at Superior north, although data from one of the two aerial temperature sites at Manitoulin (Misery Bay) could not be tested due to missing data.

4.2 Plant growth and senescence

Soil temperature and root growth strategy affected shoot dry mass. Temperature was negatively related to shoot dry mass in spring; in fall, shoot dry mass of species with single-season roots (sSSR) increased, and shoot dry mass of species with multi-season roots (sMSR) decreased. There was a positive relationship between root length and soil temperature at midseason and for maximum growth. There were significant relationships between shoot dry mass and root length in spring and midseason with harvest date, species, and temperature.

The hypothesis that the need to produce new roots in spring delays plant growth was not conclusive with respect to shoot growth, but was suggested by the nearly significant effect ($p = 0.053$) of root growth strategy on the ratio of spring to maximum shoot dry mass, with sSSR lower than sMSR. The prediction that sSSR would experience delayed shoot growth early in the growing season due to the need to produce new roots was similarly suggested. Together with the

later shoot senescence of sSSR, this suggests that growth of sSSR can be limited if the growing season is too short. The prediction that sMSR would invest in late-season root production to a greater degree than sSSR was not confirmed.

In the context of whole-plant resource economics, the use of a multi-season versus a single-season root growth strategy signals the adaptability of species in maximizing resources over the course of the growing season, given adaptations owing to differences in species phylogeny. Results of this study show that soil temperature plays an important role in the production of roots and shoots, as both root growth and shoot growth were affected by soil temperatures. Rising temperatures are clearly linked to the global increase in atmospheric carbon (e.g., Drost et al. 2012), with resulting effects on vegetation (e.g., Gorissen et al. 2004). In a review of ecosystem response to a global increase in carbon dioxide, Bazzaz (1990) indicated that there is a general increase in allocation to root growth, especially under conditions of limited nutrients and water.

The growing season of arctic plant communities has been shown to be 1.5 times longer belowground than above (Blume-Werry et al. 2016). Nord and Lynch (2009) demonstrated that root length duration is important for phosphorus acquisition. It has been acknowledged that, at high latitudes, to some extent, root production is possible in the absence of concurrent photosynthesis and that root phenology is specific to plant functional type, such as deciduous herbs or evergreen dwarf shrubs (Sloan et al. 2016). Furthermore, internal controls over carbon allocation to roots are an important driver of phenology, as evidenced by the asynchronous timing of root and shoot growth in Mediterranean plants (Abramoff and Finzi 2015).

With respect to root growth in particular, temperature was the more important factor driving root growth, compared to strategy. As expected, roots of sSSR experienced lower root growth early in the growing season (July 2) as compared to roots of sMSR, but the difference was not significant. Early in the growing season, there was a significant negative relationship between shoot dry mass and soil temperature, which was not expected. By mid-season (August 18), there was no relationship between shoot dry mass and temperature. In general, root length was positively associated with soil temperature, with the relationship being significant at midseason (and for maximum growth, which will be discussed later). Together with the negative relationship between shoot dry mass and soil temperature (in spring), this may indicate that biomass allocation to roots in spring is reduced by low soil temperatures. The *E. palustris* site at Superior north demonstrated the coldest temperatures, but root length values were highest at this site at 4 of 5 harvests, which could be due to slow decomposition. The relationship between midseason root length and soil temperature was tested both with and without data from *E. palustris* at Superior north, and were significant only when data were excluded. The different behaviour of this site justifies treating this data point as an outlier.

In their review of studies on whole-plant phenology, Abramoff and Finzi (2015) noted a positive correlation between temperature and the growth of roots and shoots in boreal and temperate biomes. Root growth results from the present study match those findings at midseason and for maximum growth (with the exception of outliers at the Superior north *E. palustris* site). Kikuzawa et al. (2013) found that leaf longevity and mean annual temperature were positively correlated for deciduous species but negatively correlated for evergreens. In their review of global root turnover, Gill and Jackson (2000) found that root turnover rates increased

exponentially with mean annual temperature in grasslands, forests, and shrublands and decreased from tropical to high-latitude systems.

The relative change in root length during the last study interval of the growing season (i.e., fall) was positive (i.e., increased) at most sites. Growth of new roots late in the season was more pronounced in sMSR than sSSR, which could in some cases be attributed to warmer sites, but the difference between the strategies was not significant. This indicates that species of both strategy types continued to invest in acquisitive capacity in below-ground resources, and that sSSR had not yet shifted toward a conservative mode of growth, i.e., senescence or cessation of growth. A later harvest would have been necessary to capture the timing of this shift. Nevertheless, together with the significantly higher investment of sSSR in acquisitive capacity of above-ground resources during the same period, this suggests that sSSR and sMSR indeed differ in their late season resource investment strategy.

The relative change in shoot dry mass at the end of the growing season was affected by root growth strategy, decreasing on average for all sMSR and increasing on average for all sSSR. For sMSR, this suggests early shoot senescence and a resource conservative strategy, with the benefit of avoiding early frost. By contrast, the late-season increase in shoot mass of sSSR indicates a resource acquisitive strategy, and suggests that the growth of sSSR would be more constrained than sMSR when the growing season is short. These observations point to the greater importance of early leaf senescence for sMSR as they must remobilize nutrients to sustain themselves over the winter, as their roots remain alive. The findings also reflect the differences between strategy types in the partitioning of resources prior to the onset of winter.

Compared to sMSR, shoot growth of sSSR was lower early in the growing season (differences not significant), but continued for a longer portion of the fall growing season. This could signal the need for the shoots of sSSR to allocate more resources to shoot production, perhaps for seed production in particular. In addition, sSSR have been shown to persist at warmer sites than sMSR (Gagnon et al. 2015), as well as produce roots with higher specific root length, *i.e.,* longer roots per unit of dry mass (Gagnon 2014); it is thus evident that there are distinct root growth strategies with varying phenological adaptations.

There was a significant positive relationship between soil temperature and maximum root length, (the greater root length value on August 18 or September 30). The relationship between maximum root length and soil temperature was tested both with and without data from *E. palustris* at Superior north, and were significant only when data from that were excluded. Shoot dry mass maximum was negatively related to GDD, but the effect was not significant ($p < 0.11$). There were no relationships between strategy and root length maximum, nor between strategy and shoot dry mass maximum.

The ratio of spring to maximum shoot dry mass was nearly significantly influenced by root growth strategy ($p = 0.053$), and was greater for sMSR, but was not influenced by temperature. This supports the hypothesis that the production of new roots in spring, which is required of sSSR, delays plant growth, at least for shoots, and matches findings of Gagnon et al. (2015). There were no relationships between the ratio of spring to maximum root length with either strategy or temperature, but the higher spring to maximum root length of sMSR may indicate a more resource acquisitive strategy than sSSR in spring.

There were significant relationships between root length at days 183 and 230 and harvest date, species, and GDD, as well as with the interaction factors harvest date × GDD and harvest date × species × GDD. There was a positive relationship between root length and GDD, but the significant interactions indicate that there is some variation in this relationship depending on species and harvest date. There were significant relationships between shoot dry mass at days 183 and 230 and harvest date, species, and GDD, as well as with the interaction factor species × GDD. There was a negative relationship between shoot dry mass and GDD, but the significant interaction indicates that there is some variation in this relationship depending on species.

These results align with those of another study of root turnover strategies of some of the same species that found that sSSR demonstrated higher above-ground relative growth rates than sMSR (Gagnon 2014). Correspondingly, Gagnon (2014) also found lower dry matter content in leaves and roots of sSSR compared to sMSR, a characteristic known to be associated with high relative growth rate, fast turnover, and an acquisitive resource strategy (Freschet et al. 2012).

In this study, the higher root length produced by 2 of the 3 sMSR (*C. lasiocarpa* and *E. palustris*) compared to root length of sSSR signals the potential for higher root production over the course of the growing season when roots survive the winter and are already present at the beginning of the season. The remaining sMSR, *T. cespitosum*, is a species of small stature (Flora of North America Editorial Committee 1993). It exhibits slow growth and is a stress-tolerant glacial relict species, sometimes referred to as an arctic alpine species, that inhabits unproductive sites (Given and Soper 1981), such as challenging coastal environments. This slow growth is characteristic of a stress-tolerant species with limited competitive traits sensu Grime (1977). The

growth form of this species may account for the lack of significant differences in root length between the two root growth strategies.

The results of this study demonstrate that there are trade-offs in survival strategy in different habitats. Resource acquisitive plants are normally associated with fast growth rate, short leaf and root lifespans, and nutrient-rich sites (Ryser 1996). It is therefore possible that sSSR maximize resource acquisition to a greater extent than sMSR, achieved via later shoot senescence and lower dependence on nutrients such as nitrogen and phosphorus remobilized from leaves.

4.3 Study limitations

This study was labour-intensive, which limited the number of replicates. Variation included soil conditions and root growth. In addition, ecological fieldwork is prone to small-scale site variation, experimental error, and outlying data. During installation of ingrowth cores, non-decomposed roots that were too small to be eliminated by the sieving process may have created artefacts in root length data. As noted by Lukac (2012), in many field studies using root ingrowth cores, despite attempts to approximate the original soil profile as much as possible, the growth environment is often dissimilar. In addition, as roots colonising the core were initially free of competitors, this may have accelerated growth and led to an overestimation of root production. Excessive root growth may also have resulted from any injuries and cuts to plants generated during the core installation process (Lukac 2012). However, it is possible that species with multi- and single-season roots may have been influenced to a similar degree, minimizing artefacts.

Harvests at Superior and Manitoulin were completed in succession, and not simultaneously, which increased error variation as harvest dates were not directly comparable. By calculating values for average harvest dates, this problem was partially, but not fully, corrected. It is also possible that some roots from non-target species were incorrectly included in root samples, despite careful assessment (visual and in some cases with a microscope).

The analysed root length may not accurately reflect the amount of living roots, as TTC did not stain living roots in a reliable manner, which is why this variable was not statistically analyzed. For roots from Harvest 2, an attempt was made to separate living roots from dead roots by visual assessment, but this proved to be an unreliable method. Due to this attempt, data from Harvest 2 was not statistically analysed.

Results indicate that certain trends in root growth strategy exist, but differences between strategies were often not significant. This could be attributed to small sample sizes in combination with large variation typical of field conditions. Improvements to data collection could be achieved by including more replicates at more sites, an equal number of multi- and single-season species at both study regions, and more study species overall. Additional root ingrowth cores and temperature dataloggers would be helpful in case the need for backup samples arises.

4.4 Study importance and future directions

This research strengthens the body of knowledge relevant to the established Leaf Economics Spectrum (Diaz et al. 2016), as well as the emerging Root Economics Spectrum (Roumet et al.

2016), Plant Economics Spectrum (Freschet et al. 2010), and Plant Community Economics Spectrum (Pérez-Ramos 2012, Prieto et al. 2015). Above- and below-ground traits such as leaf and root longevity, nitrogen content, and respiration rates contribute to plant resource economics and allow for adaptations to environmental conditions (Tjoelker et al. 2005). Species that employ a resource acquisitive strategy tend to have high Specific Leaf Area, low stem density, low resource allocation to roots, high nitrogen levels in roots, and high root nutrient uptake rates; species employing a resource conservative strategy maximise leaf and root lifespan (Poorter et al. 2014).

Expanding the body of knowledge of root growth strategies will help formalize and enhance the concept of a Root Economics Spectrum or RES, as has been done for leaves. The RES is a useful concept as it may identify useful species to include when solving problems such as the overabundance of atmospheric carbon, repairing damaged wetlands, and designing landscape features such as artificial wetlands for flood prevention. For example, the usefulness of a species for erosion control will depend on the lifespan and strength of its roots.

As research on wetland plants and root growth evolves, the knowledge gained can be applied to study why plants grow where they grow, impacts of increased CO_2 on the root environment, and the impact of roots on soil structure and stability (Atkinson 2000). Given that climate change may lead to fluctuations in carbon cycling and species distribution, it is important to understand consequences and implications of contrasting root growth strategies described in this study. These strategies may be as ecologically significant as strategies of deciduous and evergreen leaf growth observed in woody species.

In light of changing vegetation dynamics brought about by climate change, it is essential that we formalize the concept of Root and Plant Economics Spectra. Beyond this, we must understand how species functional traits relate to ecosystem level processes.

Chapter 5: Conclusion

(1) Soil and aerial temperature differed significantly among the study zones, with Manitoulin demonstrating warmer temperatures than Superior. (2) For shoot growth, both soil temperature and root growth strategy affected shoot dry mass. Early season shoot dry mass was negatively related to soil temperature. Late in the growing season, SM of sSSR increased, while SM of sMSR decreased. This indicates that shoots of sSSR continued to grow for a longer duration than those of sMSR, possibly to maximise photosynthesis before the end of the limited growing season and to translocate photosynthates directly into storage organs. The ratio of spring to maximum shoot dry mass was nearly significant (higher for sMSR, indicating delayed shoot growth for sSSR). There were significant relationships between SM at days 183 and 230 and harvest date, species, and GDD, as well as with the interaction factor species × GDD. The variation in the relationship between SM and GDD was dependent on species. (3) For root growth, there was a positive relationship between root length and soil temperature, with significant effects at midseason and for maximum growth. There was no relationship between root length and root growth strategy. Notably, the coldest sites were occupied by sMSR, but as these species also occupy warmer sites, differences between sMSR and sSSR were rendered insignificant. Throughout the growing season, root length was higher for sMSR than sSSR, but field variation was too large to provide reliable conclusions. Trends indicated that the ratio of spring to maximum root growth was greater for sMSR than for sSSR, and that at the end of the growing season, sMSR invested relatively more resources in roots than sSSR. There were significant relationships between RL at days 183 and 230 and harvest date, species, and GDD, as well as with the interaction factors harvest date × GDD and harvest date × species × GDD. The

variation in the relationship between RL and GDD depended on species and harvest date. (4) Taken together, the positive relationship between root length and soil temperature (midseason and maximum growth), and the negative relationship between spring shoot dry mass and soil temperature, suggest that biomass allocation to roots in spring is reduced by low soil temperatures. (5) With respect to shoot growth, significant results indicate that sMSR emphasize resource conservation, while sSSR emphasize resource acquisition. With respect to root growth, there were general trends supporting the idea that sMSR emphasize acquisition while sSSR emphasize resource conservation; but due to large field variation, differences between sMSR and sSSR were not significant. For conclusive results, field investigation of root behaviour would require a larger number of replicates. (6) Effects of temperature and strategy on root and shoot growth vary throughout the growing season.

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Appendices

Appendix 1. Life history information pertaining to study species.

Species	Life history information
Carex lasiocarpa	Carex lasiocarpa is a dominant of boreal wetlands, often forming huge stands. Large stands of the species are quite striking at a distance because of their pale straw color derived from the dried and faded, curly, filiform leaf apices of the vegetative shoots. Sometimes extensive stands occur without fertile culms (E-Floras, *Carex lasiocarpa*).
Eleocharis palustris	Grazed by big game animals and waterfowl, produces nesting cover for waterfowl, has utility for erosion control, constructed wetland system applications, wetland creation and restoration, increases plant diversity in wetland and riparian communities, and has bacterial associates that can fix atmospheric nitrogen and make it available to other plants in the wetland community (Tilley and St. John, 2012).
Rhynchospora fusca	Achenes are eaten by waterfowl but do not make up a large portion of their diet. Fen indicator (Newmaster et al. 1997).
Sparganium americanum	Leaves are alternate, stiff and erect or limp and floating, linear, and internally septate. Individual flowers small, in separate male or female clusters on the same plant. Among other *Sparganiaceae* members, widely distributed in temperate latitudes of eastern North America. Grows best on wet ground in rich soil, preferring full sun but tolerating some shade.
Sparganium emersum	The species typically occurs in the margins of lakes, swamps, and in the slower reaches or backwaters of large lowland rivers, it will also occasionally occur in canals and smaller ponds. It is most frequently found in mesotrophic to eutrophic systems in deep silt and usually in fairly shallow water. Considered a poor competitor and this is why it is not more often dominant (Allen 2001). Note: *Sparganium americanum* and *S. emersum* are closely related species. The study species at Superior sites 1 and 6 is *S. americanum*, while at Manitoulin site 13, the study species is *S. emersum*. Genetic verification of species was not completed as part of the present research.
Sagittaria latifolia	Offers food and cover for aquatic animal life, the seed and tubers are eaten by waterfowl, songbirds, wading birds, muskrats, and beaver, and nutrients and metals are extracted from sediments and water (USDA NRCS, 2002).
Trichophorum cespitosum	Although it is primarily a rich fen species in northern Ontario, in eastern Canada, it grows in more acidic habitats, including bogs. At the southern edge of its range, it is restricted to areas influenced by the cold Lake Superior microclimate (Newmaster et al. 1997). Considered an arctic-alpine species (Given and Soper 1981). As plant responses to changes in temperature are of global interest, this species could be of particular importance in understanding species behaviour within a limited climatic range in response to a changing climate.

Appendix 2. Numbered study sites and species under investigation at Lake Superior Provincial Park and Manitoulin Island.

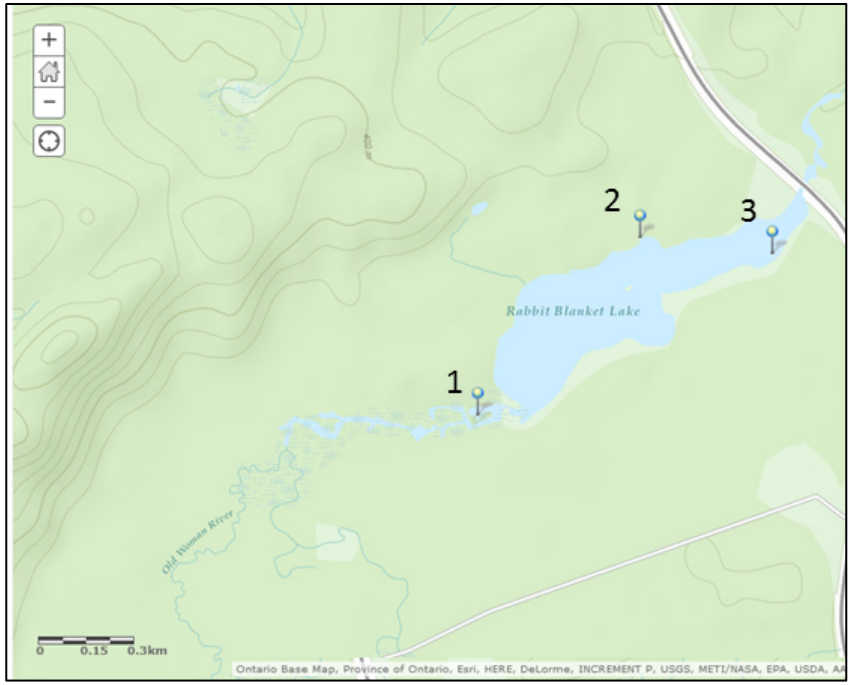

Figure A1. Lake Superior Provincial Park: *S. americanum* (1), *S. latifolia* (2), *E. palustris* (3).

Figure A2. Lake Superior Provincial Park: *C. lasiocarpa* (4).

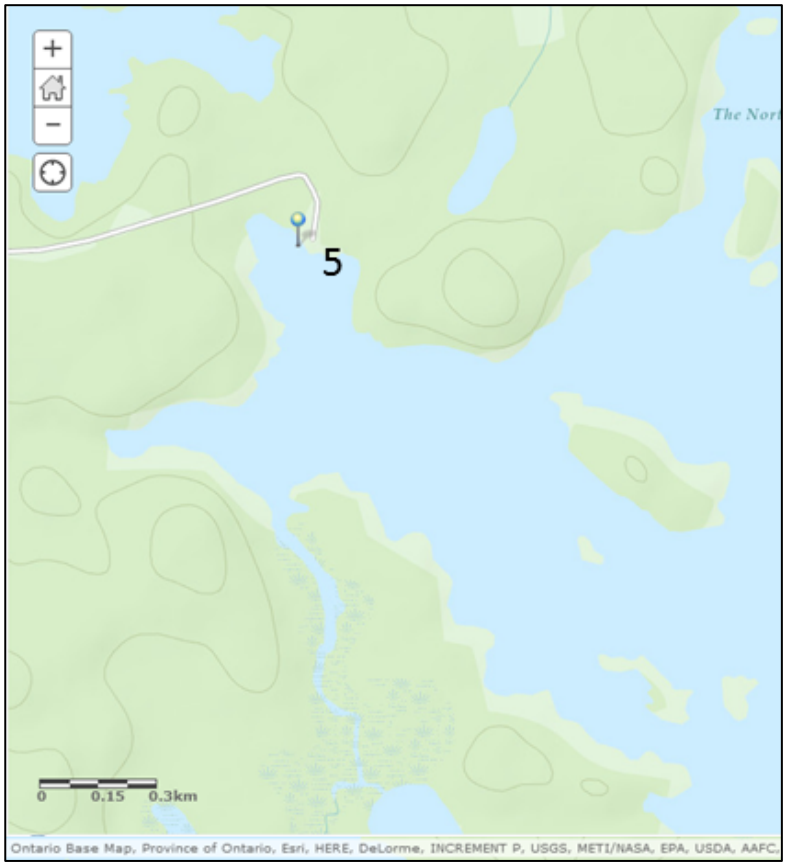

Figure A3. Lake Superior Provincial Park: *R. fusca* (5).

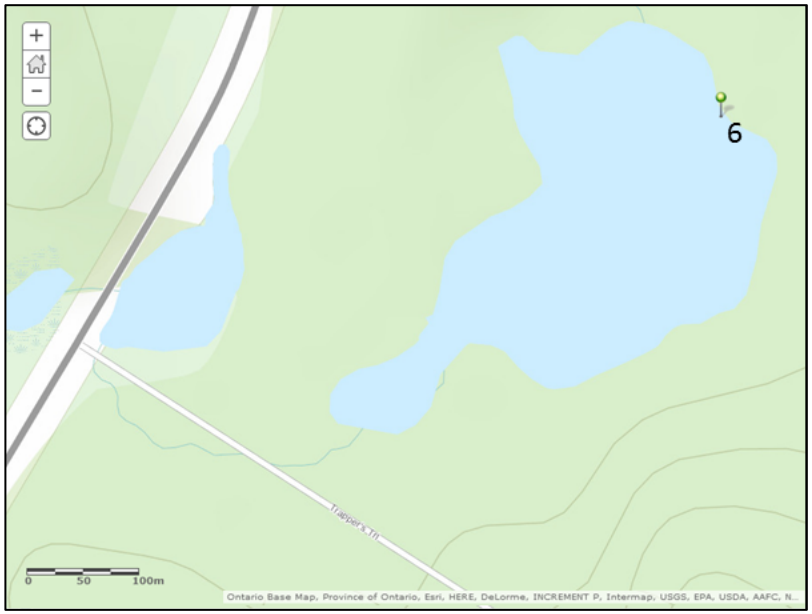

Figure A4. Lake Superior Provincial Park: *S. americanum* (6).

Figure A5. Lake Superior Provincial Park: *T. cespitosum* (7).

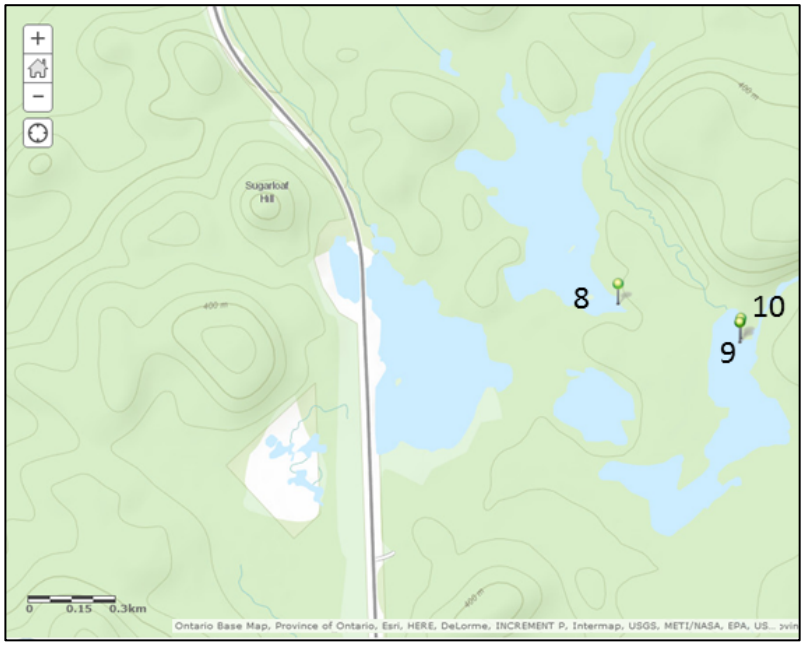

Figure A6. Lake Superior Provincial Park: *C. lasiocarpa* (8), *E. palustris* (9), *R. fusca* (10).

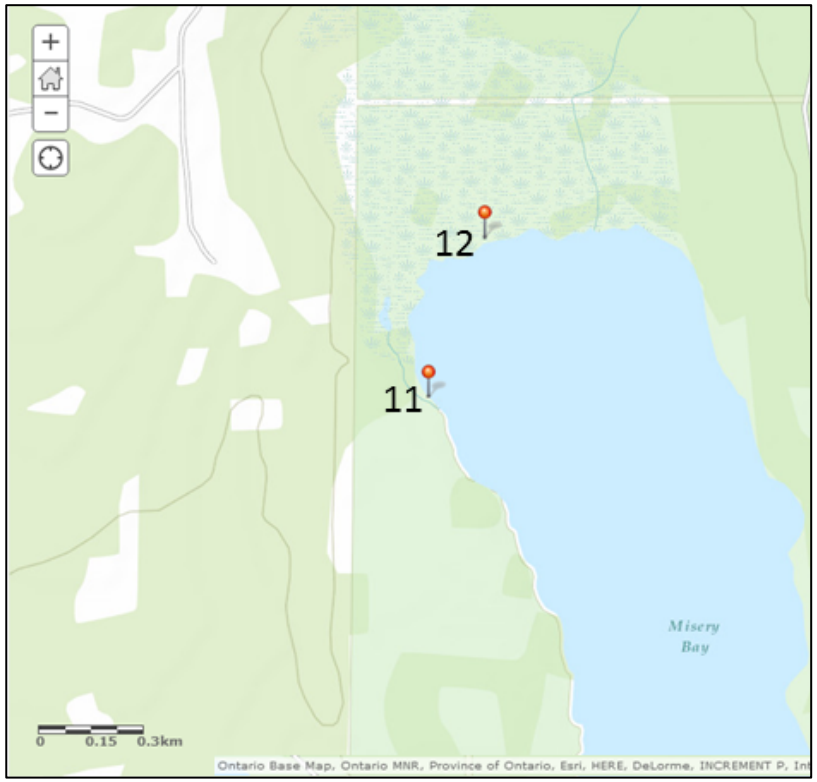

Figure A7. Manitoulin Island: *C. lasiocarpa* (11), *T. cespitosum* (12).

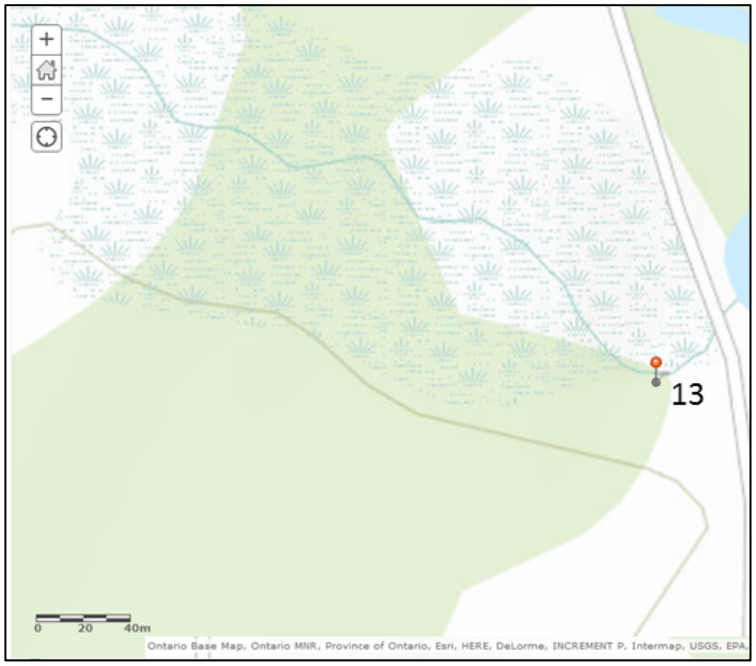

Figure A8. Manitoulin Island: *S. emersum* (13).

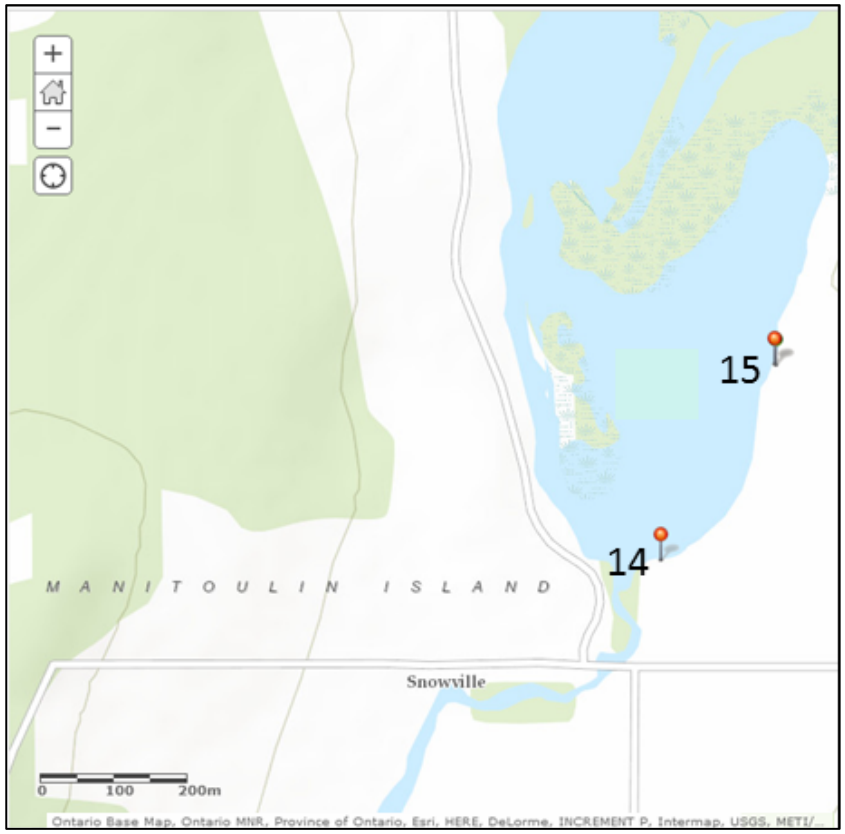

Figure A9. Manitoulin Island: *E. palustris* (14), *S. latifolia* (15).

Appendix 3: Soil parameters: pH and redox

Average pH in the top soil stratum was considerably more basic at Manitoulin (6.1) than at Superior (5.1). Average pH at sites of species with multi-season roots and at sites of species with single-season roots was 5.5 in both cases. The highest pH was observed at *S. emersum* at Manitoulin (6.9), and the lowest pH was observed at *S. americanum* and *C. lasiocarpa* at Superior south (4.7). The average redox value for sites of species with multi-season roots was -59.0, while it was -85.1 for sites of species with single-season roots. The highest positive redox values were both observed at Superior, in the southern zone: *R. fusca* at 255.1 mV and *T. cespitosum* at 241.7 mV. The lowest negative redox values were both observed at Manitoulin: *E. palustris* at -357.3 mV and *S. emersum* at -339.8 mV. The average redox value at Manitoulin was -89.0 mV, compared to -27.9 mV at Superior. Redox potential was lower at Manitoulin than at Superior and lower for species with single-season roots than for species with multi-season roots. Levels of pH were equal for multi- and single-season species, but were much higher at Manitoulin than at Superior, likely owing to differences in the parent material—limestone at Manitoulin and granite/greenstone at Superior. Comparatively acidic soil conditions at Superior could account for nutrient limitations there. Plants that are nutrient deficient may increase nutrient uptake by increasing root production later in the growing season (Radville et al. 2016). Schläpfer and Ryser (1996) and Ryser (1996) found that grass species adapted to low nutrient availability produced greater biomass over the long term.

Appendix 3. Average soil pH and redox values (mV).

Site	Strategy	Species	pH upper soil layer	pH lower soil layer	redox
1	Single	*S. americanum*	5.4	5.5	-98.1
2	Single	*S. latifolia*	5.0	5.0	-203.4
3	Multi	*E. palustris*	5.5	5.8	-198.6
4	Multi	*C. lasiocarpa*	5.1	n/a	-260
5	Single	*R. fusca*	5.4	n/a	90.5
6	Single	*S. americanum*	4.7	4.7	-47.0
7	Multi	*T. cespitosum*	5.6	n/a	241.7
8	Multi	*C. lasiocarpa*	4.7	4.7	-226.5

Site	Strategy	Species	pH upper soil layer	pH lower soil layer	redox
9	Multi	*E. palustris*	5.0	n/a	167.7
10	Single	*R. fusca*	4.9	n/a	255.1
11	Multi	*C. lasiocarpa*	5.6	n/a	19.8
12	Multi	*T. cespitosum*	6.6	n/a	141.4
13	Single	*S. emersum*	6.9	6.9	-339.8
14	Multi	*E. palustris*	6.2	6.9	-357.3
15	Single	*S. latifolia*	6.1	6.4	-252.9
		Superior	5.1	5.1	-27.9
		Manitoulin	6.1	6.7	-89.0
		Multi	5.5	5.8	-59.0
		Single	5.5	5.7	-85.1

Upper soil layer: top 5-10 cm. Lower soil layer: below 10 cm. Where soil profile below 10 cm was visually similar to top profile, only samples from top were collected and measured for pH. n=3, 1 for pH, redox per site, respectively.

Appendix 4. Sample sizes across all sites, harvests, regions, and strategies.

Study region	Site number	Species	Species code	Root length sample size	Root length sample size at harvest (H)					Shoot dry mass sample size	Shoot dry mass sample size at harvest (H)			
					H1	H2	H3	H4	H5		H1	H2	H3	H4
Lake Superior Provincial Park (north)	1	*Sparganium americanum*	SEM	20	4	4	4	4	4	37	7	10	10	10
	2	*Sagittaria latifolia*	SLA	18[a]	4	4	4	2	4	16	3	5	5	3
	3	*Eleocharis palustris*	EPA	20	4	4	4	4	4	37	7	10	10	10
	4	*Carex lasiocarpa*	CLA	20	4	4	4	4	4	37	7	10	10	10
	5	*Rhynchospora fusca*	RFU	20	4	4	4	4	4	37	7	10	10	10
Lake Superior Provincial Park (south)	6	*Sparganium americanum*	SEM	20	4	4	4	4	4	37	7	10	10	10
	7	*Trichophorum cespitosum*	TCA	18[b]	4	4	4	3	2	37	7	10	10	10
	8	*Carex lasiocarpa*	CLA	15[c]	3	3	3	3	3	37	7	10	10	10
	9	*Eleocharis palustris*	EPA	20	4	4	4	4	4	38	8	10	10	10
	10	*Rhynchospora fusca*	RFU	21*	4	4	5	4	4	37	7	10	10	10
Manitoulin Island	11	*Carex lasiocarpa*	CLA	20	4	4	4	4	4	40	10	10	10	10
	12	*Trichophorum cespitosum*	TCA	20	4	4	4	4	4	45	10	10	15	10
	13	*Sparganium emersum*	SEM	15[d]	3	3	3	3	3	37	7	10	10	10
	14	*Eleocharis palustris*	EPA	20	4	4	4	4	4	30	0	10	10	10
	15	*Sagittaria latifolia*	SLA	17[e]	4	4	4	4	1	18	3	5	5	5
			CLA	55	11	11	11	11	11	114	24	30	30	30
			EPA	60	12	12	12	12	12	105	15	30	30	30
			TCA	38	8	8	8	7	7	82	17	20	20	20
			Multi-season	153	31	31	31	30	29	301	56	80	80	80
			RFU	41	8	8	9	8	8	74	14	20	20	20
			SEM	55	11	11	11	11	11	111	21	30	30	30
			SLA	35	8	8	8	6	5	34	6	10	10	8

Study region	Site number	Species	Species code	Root length sample size	Root length sample size at harvest (H)					Shoot dry mass sample size	Shoot dry mass sample size at harvest (H)			
			Single-season	131	27	27	28	25	24	219	41	60	60	58
			Superior	192	39	39	40	36	37	350	67	95	95	93
			Manitoulin	92	19	19	19	19	16	170	30	45	45	45
			Harvest totals		58	58	59	55	53		97	140	145	138
			Grand total	283						520				

Appendix 5. Average soil and aerial temperature (Growing Degree Days) January – December, 2014, by study zone.

	Soil GDD			Aerial GDD		
	Superior north	Superior south	Manitoulin	Superior north	Superior south	Manitoulin
January	0	0	0	0	0	0
February	0	0	0	0	0	0
March	0	0	0	0	1	0
April	1	0	1	10	10	16
May	35	60	77	133	133	177[1]
June	239	292	287	313	292	321
July	315	376	360	290	257	347
August	318	378	380	322	311	372
September	221	290	309	193	188	249
October	108	140	203	52	64	118
November	11	12	43	2	4	7
December	4	0	0	0	0	0
2014	1251	1547	1660	1316	1257	1605

Soil: n=29, 24 for sites at species with multi- and single-season roots; 25, 18 for sites at Superior and Manitoulin, respectively.
Aerial: n=12, 4 for sites at Superior and Manitoulin, respectively.
[1] Data missing for May 13-23, 2014 from Misery Bay aerial temperature dataloggers.

Appendix 6. Average soil temperature (Growing Degree Days) January – December, 2014, by study site.

Site	Species	Soil Temperature (Growing Degree Days)												
		Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**2014**
Superior north														
1	SEM[δ]	0	0	0	0	55	306	344	347	236	108	2	0	1398
2	SLA[δ]	0	0	0	0	15	251	360	379	269	128	9	0	1412
3	EPA[α]	0	0	0	0	50	205	197	183	122	60	1	0	817
4	CLA[α]	1	0	0	5	30	152	335	336	229	98	28	18	1232
5	RFU[δ]	0	0	0	0	22	279	340	344	250	146	14	0	1395
Superior south														
6	SEM[δ]	0	0	0	0	34	262	336	344	257	127	12	0	1372
7	TCA[α]	0	0	0	0	82	297	342	399	294	152	9	0	1575
8	CLA[α]	0	0	0	0	33	239	389	288	279	105	8	0	1341
9	EPA[α]	0	0	0	0	54	305	401	438	332	186	25	0	1741
10	RFU[δ]	0	0	0	0	95	356	409	421	287	130	4	0	1703
Manitoulin														
11	CLA[α]	0	0	0	3	133	277	335	369	289	171	21	0	1598
12	TCA[α]	0	0	0	0	74	216	284	316	267	183	37	0	1377
13	SEM[δ]	0	0	0	0	85	258	320	333	276	180	28	0	1478
14	EPA[α]	0	0	0	0	47	344	443	461	372	246	57	0	1970
15	SLA[δ]	0	0	0	0	47	338	419	424	343	234	71	2	1878

[α] species with multi-season roots; [δ] species with single-season roots

Appendix 7. Average aerial temperature (Growing Degree Days) January – December, 2014, by study site.

Site	Air temperature (Growing Degree Days)												
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**2014**
Superior north													
2	0	0	0	7	123	301	280	310	185	50	2	0	1257
4	0	0	0	11	135	314	297	328	194	55	3	0	1337
5	0	0	0	13	139	325	294	327	201	52	2	0	1354
Superior south													
6	0	0	0	0	127	313	265	319	179	58	3	0	1264
7 (near)	0	0	2	18	98	254	248	314	203	78	5	0	1219
8	0	0	0	11	174	309	258	300	180	55	3	0	1289
Manitoulin													
12	0	0	0	16	[a]	297	333	363	248	115	8	0	[a]
14	0	0	0	15	177	345	360	382	250	120	7	0	1655

[a] Missing value due to temperature datalogger malfunction May 13-23, 2014.

Appendix 8. Cumulative soil Growing Degree Days (GDD) by harvest.

Cumulative GDD includes the sum of temperature data from date of installation to each harvest.

Site	Species	GDD H1	GDD H2	GDD H3	GDD H4	GDD H5
1	*S. americanum*	287	703	1094	1319	1365
2	*S. latifolia*	212	605	1053	1310	1374
3	*E. palustris*	157	427	647	783	800
4	*C. lasiocarpa*	146	565	951	1207	1298
5	*R. fusca*	265	729	1116	1389	1488
6	*S. americanum*	144	635	1088	1303	1382
7	*T. cespitosum*	187	796	1234	1522	1586
8	*C. lasiocarpa*	249	762	1083	1225	1288
9	*E. palustris*	316	865	1417	1679	1801
10	*R. fusca*	449	1025	1553	1763	1830
11	*C. lasiocarpa*	69	629	1356	1594	1646
12	*T. cespitosum*	90	520	1148	1390	1447
13	*S. emersum*	111	568	1241	1478	1526
14	*E. palustris*	190	812	1716	2043	2124
15	*S. latifolia*	511	670	1506	1812	1910
	Superior	212	670	1071	1294	1362
	Manitoulin	194	640	1393	1663	1730
	Multi Superior	211	683	1066	1283	1355
	Single Superior	214	648	1078	1311	1374
	Multi Manitoulin	116	654	1407	1675	1739
	Single Manitoulin	311	619	1373	1645	1718
	Multi	175	672	1194	1430	1499
	Single	283	705	1236	1482	1554

Appendix 9. Statistically analyzed shoot, root, and temperature values at average dates of harvest at each site; average values below.
$_C$ Cumulative GDD (Jan 1) ^{M} Multi-season species ^{S} Single-season species. Variables are as follows: day 183, July 2; day 230, August 18; days 230-273, relative change Aug 1–Sep 30; MAX, higher value Aug. 18 or Sep 30; 183:MAX, ratio of early to maximal growth. Replicates in Appendix 4.

Site	Strategy	Shoot dry mass (mg)					Root length (cm)					Soil Temperature (Growing Degree Days)		
		183	230	230-273	MAX	183 : MAX	183	230	230-273	MAX	183 : MAX	183	230_C	273_C
1	*S. americanum* ^{M}	634	2115	0.192	2521	0.3	322	1320	-0.027	1320	0.2	398	918	1302
2	*S. latifolia* ^{S}	206	351	3.136	1452	0.1	65	155	0.044	162	0.4	286	789	1215
3	*E. palustris* ^{M}	266	345	-0.235	345	0.8	1811	3986	0.010	4026	0.4	264	547	736
4	*C. lasiocarpa* ^{M}	725	1363	-0.178	1363	0.5	841	608	1.337	1422	0.6	163	556	937
5	*R. fusca* ^{S}	41	68	0.000	68	0.6	546	900	0.107	996	0.5	323	843	1237
6	*S. americanum* ^{S}	548	1392	0.417	1973	0.3	528	1353	0.192	1613	0.3	317	829	1230
7	*T. cespitosum* ^{M}	45	69	-0.175	69	0.6	121	256	2.155	808	0.1	401	944	1403
8	*C. lasiocarpa* ^{M}	319	617	-0.027	617	0.5	1027	1811	-0.098	1811	0.6	292	776	1107
9	*E. palustris* ^{M}	177	317	0.096	348	0.5	512	1106	0.369	1514	0.3	384	1011	1526
10	*R. fusca* ^{S}	27	42	0.122	47	0.6	221	831	0.267	1053	0.2	478	1101	1565
11	*C. lasiocarpa* ^{M}	344	330	-0.091	330	1.0	1517	2164	0.281	2772	0.5	433	961	1401
12	*T. cespitosum* ^{M}	71	96	0.027	98	0.7	151	194	0.780	345	0.4	306	756	1153
13	*S. emersum* ^{S}	178	183	0.453	266	0.7	239	475	0.753	833	0.3	362	850	1262
14	*E. palustris* ^{M}	–	286	-0.214	286	–	806	1225	0.209	1481	0.5	418	1101	1661
15	*S. latifolia* ^{S}	133	777	0.311	1018	0.1	677	526	-0.242	526	1.3	388	1048	1562
	Superior	236	348	0.048	482	0.5	520	1003	0.149	1371	0.4	320	836	1234
	Manitoulin	156	286	0.027	286	0.7	677	526	0.281	833	0.5	388	961	1401
	Multi Superior	266	345	-0.175	348	0.5	841	1106	0.369	1514	0.4	292	776	1107
	Single Superior	206	351	0.192	1452	0.3	322	900	0.107	1053	0.3	323	843	1237
	Multi Manitoulin	207	286	-0.091	286	0.9	806	1225	0.281	1481	0.5	418	961	1401
	Single Manitoulin	156	480	0.382	642	0.4	458	501	0.256	680	0.8	375	949	1412
	Multi	266	324	-0.133	337	0.6	823	1166	0.325	1498	0.5	345	860	1277
	Single	178	351	0.311	1018	0.3	322	831	0.107	996	0.3	362	850	1262

Appendix 10. Median Root Length (RL; cm) values in ingrowth cores (340 cm^3) of all study species at each site at each harvest, harvest dates with average values for species with multi- and single-season roots and for sites at Superior and Manitoulin and harvest dates. Replicates in Appendix 4.

Site[a]	Strategy	Species	Date H1[b]	RL H1	Date H2	RL H2	Date H3	RL H3	Date H4	RL H4	Date H5	RL H5
1	Single	*S. americanum*	176	176	214	1207	250	1722	286	1046	137	1846
2	Single	*S. latifolia*	176	51	211	53	250	192	286	113	137	119
3	Multi	*E. palustris*	167	1082	209	1223	248	4773	290	3535	137	5135
4	Multi	*C. lasiocarpa*	161	948	209	372	246	534	289	1931	127	1474
5	Single	*R. fusca*	167	416	210	260	246	794	282	766	136	1776
6	Single	*S. americanum*	162	165	210	276	252	1721	283	1564	136	938
7	Multi	*T. cespitosum*	161	59	216	264	251	314	289	1155	127	474
8	Multi	*C. lasiocarpa*	174	879	218	984	255	2209	284	1299	128	1357
9	Multi	*E. palustris*	170	351	214	288	255	1408	284	1576	128	2935
10	Single	*R. fusca*	169	42	214	279	255	1262	284	1002	128	2078
11	Multi	*C. lasiocarpa*	128	771	198	778	263	2602	300	3212	122	6371
12	Multi	*T. cespitosum*	128	102	198	101	263	223	300	660	122	365
13	Single	*S. emersum*	144	46	198	23	263	635	300	1345	123	350
14	Multi	*E. palustris*	143	456	199	564	263	1508	300	1411	122	1744
15	Single	*S. latifolia*	186	667	199	69	263	424	300	334	123	1385
		Superior		263		330		1565		1432		1416
		Manitoulin		456		101		635		1345		1385
		Multi Superior		879		372		1408		1576		1474
		Single Superior		165		276		1721		1046		938
		Multi Manitoulin		456		564		1508		1411		1744
		Single Manitoulin		357		46		529		840		868
		Multi		613		468		1458		1494		1609
		Single		165		260		794		1002		1385

[a]Sites 1-5: Superior north, sites 6-10: Superior south, sites 11-15: Manitoulin. [b]Harvests 1 to 5 abbreviated as H1 to H5.

Appendix 11. Median Root Length (RL; cm) and median percentage of root tissue stained by TTC, by harvest.

Site	Strategy	Species	H1 RL	H1 % stained	H2 RL	H2 % stained	H3 RL	H3 % stained	H4 RL	H4 % stained	H5 RL	H5 % stained
1	Single	*S. americanum*	176	83	1207	98	1722	98	1046	95	1846	100
2	Single	*S. latifolia*	51	76	53	98	192	97	113	92	119	100
3	Multi	*E. palustris*	1082	86	1223	94	4773	99	3535	100	5135	100
4	Multi	*C. lasiocarpa*	948	88	372	97	534	92	1931	95	1474	97
5	Single	*R. fusca*	416	95	260	98	794	98	766	99	1776	100
6	Single	*S. americanum*	165	85	276	85	1721	92	1564	93	938	97
7	Multi	*T. cespitosum*	59	100	264	99	314	98	1155	98	167	100
8	Multi	*C. lasiocarpa*	879	78	984	96	2209	89	1299	74	1357	99
9	Multi	*E. palustris*	351	88	288	85	1408	96	1576	94	2935	100
10	Single	*R. fusca*	42	88	279	97	1262	95	1002	89	2078	100
11	Multi	*C. lasiocarpa*	771	95	778	99	2602	100	3212	99	6371	100
12	Multi	*T. cespitosum*	102	100	101	98	223	100	660	100	365	100
13	Single	*S. emersum*	46	78	23	93	635	97	1345	96	350	97
14	Multi	*E. palustris*	456	89	564	89	1508	98	1411	94	1744	98
15	Single	*S. latifolia*	667	50	69	91	424	99	334	87	1385	99

Appendix 12. Median Shoot dry Mass (SM; mg) values of all study species at each site at each harvest, harvest dates with average values for species with multi- and single-season roots and for sites at Superior and Manitoulin and harvest dates. Replicates in Appendix 4.

Site[a]	Strategy	Species	Date H1[b]	SM H1	Date H2	SM H2	Date H3	SM H3	Date H4	SM H4
1	Single	*S. americanum*	176	261	214	2102	250	2130	286	2733
2	Single	*S. latifolia*	176	202	211	220	250	479	286	1980
3	Multi	*E. palustris*	167	178	209	400	248	301	290	239
4	Multi	*C. lasiocarpa*	161	322	209	1173	246	1497	289	904
5	Single	*R. fusca*	167	18	210	65	246	70	282	66
6	Single	*S. americanum*	162	84	210	929	252	1724	283	2086
7	Multi	*T. cespitosum*	161	29	216	67	251	73	289	46
8	Multi	*C. lasiocarpa*	174	259	218	604	255	641	284	577
9	Multi	*E. palustris*	170	137	214	265	255	394	284	322
10	Single	*R. fusca*	169	21	214	40	255	46	284	48
11	Multi	*C. lasiocarpa*	130	101	198	452	263	254	300	418
12	Multi	*T. cespitosum*	128	18	198	91	263	107	300	75
13	Single	*S. emersum*	144	423	198	162	263	204	300	425
14	Multi	*E. palustris*	143	0	199	386	263	185	300	329
15	Single	*S. latifolia*	186	176	199	419	263	1141	300	702
		Superior		190		502		560		740
		Manitoulin		101		386		204		418
		Multi Superior		178		400		394		322
		Single Superior		202		929		1724		2086
		Multi Manitoulin		18		386		185		329
		Single Manitoulin		300		290		673		564
		Multi		119		393		278		325
		Single		176		220		479		702

[a]Sites 1-5: Superior north, sites 6-10: Superior south, sites 11-15: Manitoulin. [b]Harvests 1 to 5 abbreviated as H1 to H5.

Appendix 13: Garden experiment

In addition to the field experiment, study species were planted in an experimental garden in Azilda, Ontario. The purpose was to grow species from different sites in controlled conditions and determine whether species provenance accounts for differences in root growth. Specimens of each study species were obtained from all sites in fall of 2013 (Superior's northern and southern zones and Manitoulin). Five replicates of each species from Superior and five from Manitoulin were planted on May 29-30, 2014 in potting soil (President's Choice® Black Earth Topsoil; Loblaws, Brampton, ON, Canada), after sieving to remove any roots present in the growing medium, in 10 L pots with holes in the bottom. Only *T. cespitosum* was planted in a mixture of peat and 1% composted manure, to simulate natural growth conditions, which differ from those of the other species. The pots were placed in beds that were kept flooded with water, which created saturated conditions similar to those in the wetlands in which the plants grow naturally. Roots were thus completely submerged in water for the duration of the experiment. Two root ingrowth cores were placed in each pot in order to measure root length that grew therein. Due to time constraints imposed by ongoing field harvesting at Superior and Manitoulin, only *C. lasiocarpa, E. palustris,* and *S. emersum* roots were harvested at two harvests—half of the cores from each species were harvested on September 26, 2014 and the remaining half on November 3, 2014. Garden harvests were completed on September 26 (Harvest 1) and November 3, 2014 (Harvest 2). Results from the experimental garden showed that at the first harvest, for species with multi-season roots, RL was lower for specimens of Manitoulin origin than those of Superior origin (8% lower for *C. lasiocarpa*, 13% lower for *E. palustris*). For species with single-season roots, RL was lower for specimens of Superior origin (74% lower for *S. emersum*). At the second garden harvest, only *C. lasiocarpa* was lower at Manitoulin (23% lower), while *E. palustris* and *S. emersum* were lower at Superior (31% and 65% lower, respectively). Replicates were too few, which created outliers, thus statistical analyses on data from the garden experiment were not conducted.

Appendix 13. Median Root Length (RL; cm) of harvests at experimental garden in Azilda, Ontario.

Region	Strategy	Species	RL Garden Harvest 1	RL Garden Harvest 2
Superior	Multi	*C. lasiocarpa*	3319	4042
Manitoulin	Multi	*C. lasiocarpa*	3061	3118
Superior	Multi	*E. palustris*	6173	4809
Manitoulin	Multi	*E. palustris*	5362	6927
Superior	Single	*S. americanum*	935	968
Manitoulin	Single	*S. emersum*	3558	2747

n=5 per region and species combination, total: 30. Harvest 1: September 26, 2014; Harvest 2: November 3, 2014.

Appendix 14: Late season growth

To quantify root growth during the period of plant senescence during fall 2014, additional cores were installed at selected sites at Superior and Manitoulin during Harvest 3 (early to mid-September) and removed during Harvest 4 (mid- to late October). To ensure a controlled, root-free growing environment, sieved autoclaved peat and sand were used as cylinder substrate. At Superior, the extra cores were installed at the northern *C. lasiocarpa site* using autoclaved peat, and at the northern *E. palustris* site using local sand. At Manitoulin, the extra cores were installed at the *C. lasiocarpa* site using autoclaved peat, and at the *E. palustris* site using imported sand. Four replicates were installed at each site. Cores produced RL ranging from 241 to 988 cm, indicating that roots of species with multi-season roots continued to grow late in the growing season. Results were not statistically analyzed.

Appendix 14. Additional cores installed at selected sites at Superior and Manitoulin during Harvest 3 (early to mid-September) and removed during Harvest 4 (mid- to late October).

Site[a]	Strategy	Species	RL
3	Multi	*E. palustris*	287
4	Multi	*C. lasiocarpa*	988
11	Multi	*C. lasiocarpa*	248
14	Multi	*E. palustris*	241

[a]n=4 per site, total: 16.

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Ministry of Natural Resources and Forestry
April 2017 – Present

Terrestrial Monitoring Biologist
Ministry of Natural Resources and Forestry
2016 – 2017

Secondary School Biology Teacher
Algoma District School Board
2012 – 2013

Management Biologist
Ministry of Natural Resources and Forestry
Feb 2017 – March 2017

Graduate Teaching Assistant Biology, Ecology
Laurentian University
2013 – 2015

Natural Heritage Educator
Ministry of Natural Resources and Forestry
2009 – 2016